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A century of spectrochemistry

Spectrochemistry effectively began a hundred years ago, with the invention of the flame spectrometer by Bunsen and Kirchhoff in 1859: this very quickly led to a whole series of outstandingly important results, not only in chemistry but in astronomy. Some of the possibilities had, however, been foreseen by earlier workers. Thus T. Melville, as early as 1752, noted the yellow colour given to flames by the introduction of sodium compounds, and A. S. Marggraf, in 1758, mentioned the lavender potassium flame. Herschel added to the list the crimson flame of strontium compounds and the green of copper compounds and borates, and suggested the possibility of detecting small quantities of elements by this method. Fox Talbot, in the 'Philosophical Magazine' in 1834, described the use of a prism to distinguish between the carmine red imparted to a flame by lithium compounds and the crimson of strontium. 'The prism displays between them the most marked distinction that can be imagined. The strontia flame exhibits a number of red rays well separated from each other by dark intervals, not to mention an orange and a bright blue ray': lithium, he recorded, gave a single red 'ray'. He, too, was impressed by the minute quantities needed for what he called 'optical analysis'. Such simple flame-tests are still standard procedure in inorganic analysis.

It was, however, Kirchhoff who established the fundamental principles of spectrum analysis: Kirchhoff was, as Roscoe put it, the most important discovery made by Bunsen during his stay at Breslau. When Joly moved to Munich, Bunsen arranged for Kirchhoff to become professor of physics at Heidelberg: the partnership thus started in 1854 was immensely fruitful.

The first flame spectrometer was constructed by Kirchhoff in 1859. The essential features were a bunsen burner—previous workers had used the less efficient spirit lamp—a hollow glass prism filled with carbon disulphide, and a simple viewing telescope (an improvement introduced by Fraunhofer) with a wavelength scale reflected into it by means of a mirror. This simple instrument was used for the identification of a long list of new elements, and also to establish the relationship between emission and absorption spectra.

Absorption spectroscopy may be said to date from Wollaston's observation of dark lines in the Sun's spectrum, though it was Fraunhofer, in 1814, who first charted these systematically. Fraunhofer noticed that a prominent black line—he called it the D line—in the Sun's spectrum appeared in the same position as the yellow line of a sodium flame, but he did not appreciate the significance of this. Kirchhoff, in 1859, showed that the dark lines could be produced by placing a sodium flame between a light source and the spectrometer slit: similarly, a sodium flame placed in front of the slit would intensify the D lines in the solar spectrum. From these observations, Kirchhoff concluded that there must be sodium in the Sun's atmosphere. It was, Bunsen wrote, a research that 'gives us sleepless nights', for the method could reveal the composition not only of the Sun but of other stars with the same certainty as chemical methods gave with terrestrial substances. Kirchhoff's memoir of that year to the Berlin Academy was concerned with the spectra of elements, the reversal of spectral lines, the constitution of the Sun's atmosphere, and physical aspects of the solar spectrum.

Kirchhoff as a physicist was ideally complemented by Bunsen as a chemist: the latter abandoned much of his other work in order to collaborate with Kirchhoff. 'Don't be angry with me, dear Roscoe, if I have still done nothing more on our photochemical work', he wrote in 1860, when they were busy at Heidelberg trying to separate a fourth alkali metal—sodium, potassium, and lithium were already known. Although they knew that their method was extremely sensitive, for they had detected lithium in twenty grains of sea water, they doubted if they would be able to isolate enough of the new element for an orthodox chemical investigation. However, they were, as Bunsen put it, 'fortunate': 'persevering' would be a more appropriate word. They evaporated to dryness forty tons of mineral water from Dürkheim, removed the alkaline earths and most of the lithium from the residue, and examined it with their spectrometer. They identified the lines of sodium, potassium, and lithium, and also two blue lines very close together. As there was no element known to give such lines, they concluded that they had discovered a new alkali metal: they called it caesium, from a Latin word for blue. They isolated 50 grams of caesium chloroplatinate from the concentrated residue, and established the principal chemical properties of the element with the 17 grams of caesium chloride that finally resulted:

twenty years later Setterberg prepared the free metal by electrolysis. Caesium was the first element whose existence was discovered spectroscopically.

Rubidium, the fifth alkali metal, was discovered very soon after caesium, the discovery being reported to the Berlin Academy in 1861. To obtain it, impure potassium platinichloride, prepared from lepidolite, was repeatedly washed with boiling water. Gradually the potassium lines in the spectrum weakened and two violet lines became stronger; new lines appeared, including the two brilliant red lines that gave the name to the new element.

The application of Bunsen and Kirchhoff's techniques quickly led to the identification of more new elements. William Crookes was keenly interested in the new technique. Needing some tellurium for a series of experiments, he decided to try to extract it from some selenium purification residues. Tests indicated the presence of tellurium in the material, but Crookes was unable to extract any and decided to look for the element spectroscopically. A portion of the residue gave a spectrum of selenium and, as this became fainter, he expected the tellurium spectrum to appear: instead, 'a bright-green line flashed into view and quickly disappeared'. Crookes was able to eliminate the presence of all but five elements from the residues; he examined the spectra of these elements and of some of their compounds and found no trace of the green line. He therefore concluded that it was due to a new element; he called it thallium, from the Greek word for a green shoot. He reported the discovery in 1861 in his 'Chemical News'.

Other new elements quickly followed. Indium was discovered by R. Reich and T. Richter in 1863 and gallium (the name is a patriotic tribute by the discoverer), samarium, and dysprosium by L. de Boisbaudran. The existence of ytterbium, holmium, and neodymium also was discovered by spectroscopy. Bunsen himself mapped the spark spectra of these and other rare-earth elements, but in these researches he was doubly unfortunate. Firstly, he was unaware of the effects on the spectra of this different form of excitation; secondly, the original manuscript was destroyed, ironically, by a fire caused by the focusing of the Sun's rays by a water-bottle.

Meanwhile a startling result had followed Kirchhoff's use of the spectroscope to reveal the presence of sodium in the Sun. The discovery started in 1868 with the identification by Janssen of a third line, which he called D_3, close to the sodium lines D and D_2, in the spectrum of the Sun's chromosphere; Lockyer—who independently identified this line a few days later—and Frankland ascribed this line to an element, unknown on the earth, which they called helium. Not for several years was helium found on the earth, although had he had greater luck Hillebrand might have made the discovery almost at once. In an investigation of cleveite, a uranium mineral, he found that on heating it an inert gas was evolved. This he wrongly identified as nitrogen—it was in fact largely helium—although in a spectroscopic examination he came very close to attributing certain spectral lines to a new element. Subsequently, Miers drew Ramsay's attention to this work and the latter identified Hillebrand's 'nitrogen' as a new gas which he tentatively called krypton. In March 1895 Ramsay left a sample with Crookes, who a week later telegraphed 'krypton is helium'. Ramsay in his turn telegraphed to Berthelot, whom he was due to meet in Paris a few days later, saying '*Gas obtenu par ma cleveite melange argon helium. Crookes identifie spectre. Faites communication academie lundi.*'

The discovery of helium in the Sun involved no new principle, but it greatly stimulated spectrometric analysis of the light of the Sun, planets, and stars, and this line of research quickly resulted in the gaining of a wealth of new astronomical knowledge of a kind that could not have been imagined in 1859. It proved capable of giving information not only about the composition of the Sun and stars but about much else beside. It confirmed, for example, that the planets shine by reflected sunlight.

In 1824 Fraunhofer found the spectrum of Sirius to be different from that of the Sun. In 1833 Brewster showed that certain of the Fraunhofer lines in the solar spectrum became stronger when the altitude of the Sun was low, while others were not affected. He thus proved that some of the Fraunhofer lines were of atmospheric origin. William Huggins made a survey of the spectra of the stars and nebulae visible from London, and it was found that they could be classified according to the characteristics of their spectra. He made, in 1867, pioneer measurements of line-of-sight velocities of a few stars from the Doppler displacement of their spectral lines, and in the same year showed, from their spectra, that some of the nebulae are gaseous. Huggins, again, was among those who suggested the use of the spectroscope for observation of the Sun's prominences at periods other than those of eclipse: in recent years this kind of research has led to important new knowledge of solar phenomena.

The biochemistry of the mammary gland

J. M. BARRY

This article reviews recent work and summarizes what is known of the biochemistry of the mammary gland when it is fully developed and secreting milk. It considers what compounds the lactating gland absorbs from the blood, what chemical reactions they undergo in the secretory cells when milk is made, and what proportion of the fat, protein, and lactose of milk, and of the energy requirements of the gland, are derived from each of these compounds.

The secretory cells of the mammary gland are all of one type and continuously make milk from components of the blood, the milk being stored within the gland until removed. The three main components of milk are fat, proteins, and milk sugar or lactose, and these differ from any of the corresponding components of blood. It also contains smaller amounts of inorganic substances and traces of many other organic compounds; the latter include vitamins, most of which are found also in blood. The milk of cows and goats usually contains 3–5 per cent each of fat, proteins, and lactose; human milk has less protein and more lactose.

Our knowledge of the biochemistry of the mammary gland is becoming more comprehensive than that about any other organ of the body. Not only do we know many enzymatic reactions that occur in the gland, but our information is quantitative, which is still rare in biochemistry. We have gone a long way towards discovering which compounds the gland absorbs from the blood and in what relative amounts, and in finding out what proportion of each of these compounds passes along each pathway of metabolism. All this is possible largely because of the structure of the gland. The blood leading to and from it can be easily tapped, and most of the products of its metabolism are not returned to the blood, as in other organs, but are secreted into the milk. As a result, quantitative experiments with isotopic tracers or by arteriovenous measurements are relatively easy to perform.

When we consider which compounds of blood could possibly be important metabolites of the mammary gland, we have, fortunately, to take account only of those whose concentration lies above a minimum which, at least for the cow and goat, can be calculated. A number of measurements show that approximately 500 litres of blood flow through the gland of a cow for each litre of milk secreted [1]. This figure is rough, but we can be almost certain that the true value is always below 1000. If we assume the value of 500 to be correct, then, if 5 g of any compound of blood is to be available for the formation of a litre of milk, its concentration in blood must be at least 10 mg per litre. Cow's blood contains around 500 mg of glucose per litre, and glucose could therefore be an important metabolite. On similar reasoning, many other compounds can be excluded. The careful analyses of W. H. Stein and S. Moore [2] show that peptides of blood can at best provide less than 10 per cent of each amino-acid of milk proteins. Propionic and butyric acids, which are absorbed in large amounts from the cow's gut, are present in such low concentrations in arterial blood that they could each provide only about 1 g for every litre of milk—an insignificant amount [3].

The metabolism of the mammary gland varies between species. The variation is most marked between ruminants and other mammals, as has been clearly shown by some experiments of S. J. Folley and his colleagues at Reading. They measured the rates at which thin slices of lactating mammary glands absorbed oxygen when suspended in salt solutions to which various organic compounds had been added. It was found that the rat's mammary gland can oxidize glucose more rapidly than it can acetate, whereas the sheep's gland oxidizes acetate more rapidly than glucose [4]. This difference between ruminants and other mammals is clearly related to their digestion: ruminants absorb large quantities of acetate from their alimentary canals but almost no glucose. We might guess that this is a genetic difference in tissue metabolism, but this does not in fact seem to be true. The evidence suggests that the difference is induced in the young ruminant by the products of digestion as its food changes from milk to herbage, and that a similar change could be induced in other animals by giving them food containing much fat and little carbohydrate.

PROTEINS

The principal proteins of milk are casein and β-lactoglobulin, and we have recently made great advances, by using isotopic tracers, in discovering which compounds the mammary gland takes from the blood to make the proteins of milk. Proteins are composed of amino acids that are of two types: essential, which, roughly speaking, cannot be made in the body, and non-essential, which can. The only possible sources of the essential amino acids of milk proteins are the same amino acids free in solution in the blood or combined in plasma proteins. T. S. Work and his colleagues in London, and my colleagues and I at Oxford, have tried to discover what proportion of the essential amino acids of casein and β-lactoglobulin come from each of these sources [5]. These experiments originated in an attempt to discover whether tissue proteins could be made from plasma proteins without intermediate hydrolysis to free amino acids. Since then, it has become clear that every protein has its own peculiar amino-acid sequence, which is usually quite unlike that in any other protein, and it is therefore highly unlikely that one protein can be converted to another without complete dismantling of the amino-acid chains. The possibility remains, however, that tissues can obtain supplies of amino acids by hydrolysing plasma proteins, and the mammary gland is one of the few organs of the body in which this possibility can be tested.

Arterio-venous measurements had previously shown that free amino acids are absorbed by the lactating mammary gland of cows and goats in significant amounts, but the concentration of proteins in blood plasma is so high that even if some were absorbed by the gland the arterio-venous difference would be undetectable. Work and I did experiments in which we injected certain essential amino acids, which had been labelled with radioactive carbon, into the blood-stream of lactating goats and rabbits. For a few hours after each injection, the free amino acids of the blood were highly radioactive, while the plasma proteins contained little activity. Casein and β-lactoglobulin obtained from milk samples taken during this time were highly active. This suggested that a significant part of their essential amino acids had come from the free amino acids of blood and not from plasma proteins, although there remained the possibility that the radioactive amino acids had been rapidly incorporated into a small fraction of the plasma proteins in the liver before being absorbed by the mammary gland. I was able to exclude this possibility by showing that the same results were obtained when an isolated mammary gland was perfused with blood. In a quantitative experiment with tracers I was able to show that at least 70 per cent of each of two essential amino acids in casein came from the same two free amino acids in blood. No experiments have disproved that plasma proteins provide a small proportion of the amino acids of casein and β-lactoglobulin, but there is no evidence in favour of it.

At Oxford we have also tried to find whether the non-essential amino acids of casein also come directly from the blood or are formed within the gland from other compounds. We found from experiments with radioactive amino acids that, in the goat, at least 70 per cent of the glutamine and the glutamic acid and at least 50 per cent of the asparagine and the proline of casein come from the same amino acids in solution in the blood. These results show, incidentally, that glutamine and asparagine are incorporated into proteins as two amino acids quite distinct from glutamic and aspartic acid. This fact is important, and is not always remembered when attempts are made to discover what sequence of bases in genetic deoxyribonucleic acid causes a particular sequence of amino acids in a protein. Experiments with labelled glucose show that less than 20 per cent of the glutamine and glutamic acid, and less than 17 per cent of the asparagine and aspartic acid, are made within the gland from blood glucose. Arterio-venous measurements also revealed a large absorption of free serine from the blood [5].

It is therefore clear that the non-essential amino acids of casein mainly come direct from the blood. The enzymes for making them do nevertheless seem to be present in the gland, for M. Kleiber and his colleagues [6] have shown that if radioactive acetate is injected into one quarter of a cow's udder, the non-essential amino acids in milk proteins from that quarter become more radioactive than those from other quarters.

Further, H. G. Wood and his colleagues have recently shown that some serine is formed from blood glucose within the gland [7]. These workers injected radioactive glucose into the artery leading to one half of the udder of a cow and, by draining away the venous blood for a few minutes, prevented almost all the glucose which was not absorbed by that half reaching other tissues. Like a perfusion, this ingenious technique allows us to study the chemistry of the gland uncomplicated by that of other tissues but, unlike a perfusion, leaves the gland functioning normally. They were able to conclude

that about 25 per cent of the carbon of serine in casein came from the carbon of blood glucose.

Tracer experiments on the other proteins of milk show that α-lactalbumin is formed, largely at least, from free amino acids of the blood, but that the immune globulin and serum albumin of milk come from the blood without change. However, the immune globulin of cow colostrum (the milk formed in the first few days of lactation) seems to be made from free amino acids by plasma cells held in the mammary gland [5].

FATS

The milk fat of most mammals, as shown by the analyses of T. P. Hilditch, has a composition very similar to that of the remainder of their body fat. That of ruminants, and of some other herbivores, however, contains a high proportion of short-chain fatty acids with 4, 6, 8, 10, 12, and 14 carbon atoms—acids which are almost entirely absent from their own fat depots and from those of other mammals. In the fat of cow's milk about 30 per cent of the fatty acid molecules have these short chains [8]. There are also, surprisingly, traces of acids with 7, 9, 11, 13, 15, and 17 carbon atoms, which are rarely found in nature [9]. We might expect to find, in cow's milk, that each triglyceride molecule contains only short- or only long-chain acids, but in fact most of them contain fatty acids of each type.

A great deal has been discovered about the compounds which the mammary gland takes from the blood to provide the fat of milk, but much still remains unclear. Firstly, it seems certain that in ruminants all, or a large part, of the long-chain stearic and oleic acids in milk fat come directly from the blood. The evidence for this is that when labelled stearic acid, either free or combined in triglycerides, was fed to cows or goats, up to 60 per cent was recovered as stearic and oleic acid in milk fat. When triglycerides containing labelled stearic acid were injected into a cow's bloodstream, a large proportion of the label was recovered in the milk fat. Further, injection of labelled glucose and acetate into a cow has shown that little stearic and oleic acids are formed in the gland from these precursors. However, in precisely what chemical form the stearic and oleic acids pass from the blood to the secretory cells is not clear, and clarification of this question might give important information about the transport of fatty acids to tissues. R. F. Glascock at Reading has recently discovered that when labelled stearic acid in triglycerides was fed to a cow it was partly carried to the milk in a small fraction of the blood which did not exchange with the bulk of the stearic acid of blood fat [10].

It is clear that part of the short-chain fatty acids of ruminant's milk are synthesized in the gland from acetate normally present in the blood of these animals. Thus, there is a significant arteriovenous difference in acetate across the glands of lactating cows. Slices of the glands of ruminants respire rapidly in acetate solutions with a high respiratory quotient—a sign of fatty acid formation—and, in fact, rapidly incorporate labelled acetate into fatty acids. The perfused mammary gland incorporates labelled acetate into the fatty acids of chain length shorter than those of stearic and oleic. The origin of these acids has been further clarified by S. J. Folley, G. Popjak, and their colleagues, who injected labelled acetate into a goat and isolated each fatty acid of even chain length from samples of milk. The specific activities of these acids from the combined milk samples differed from one another, showing that some at least were not completely derived from acetate of the blood. Almost the least active was the 4-carbon acid. Those with 6, 8, and 10 carbon atoms also contained a chain of four atoms with this low activity, but the activity of their other carbon atoms was higher. It seems almost certain that of every ten molecules of the 4-carbon acid in milk fat, four were made in the gland from blood acetate and six were made from some other compound in the blood. It also seems almost certain that the 6-, 8-, and 10-carbon acids are made completely by the addition of blood acetate to the 4-carbon acid. However, it is just possible that an equal proportion of each of these acids comes from some other compound of the blood, since the specific activity of blood acetate was not followed in this experiment. Experiments with tracers show that this other compound, if it exists, is not stearic acid, oleic acid, or glucose. The specific activities of the 14- and 16-carbon acids were equal and were only about one-third of that of the 10-carbon acid. This suggests that two-thirds of these molecules in milk come from the plentiful 16-carbon acid of blood fat, and the remaining third are built up in the gland, like the shorter-chain acids. The activity of the 12-carbon acid showed that a small portion of it must also be made in a different way from the shorter-chain acids [4]. Also interesting is that the traces of fatty acids with odd numbers of carbon atoms in milk fat arise from the combination of acetate with traces of propionic acid from the blood [9].

The origin of the glycerol of ruminant's milk fat is not clear, but H. G. Wood and his colleagues showed that in a cow about 17 per cent of the carbon of the glycerol was derived in the gland from blood glucose [7]. The fact that short-chain fatty acids, which are formed in the gland, are usually found on the same glycerol molecules as stearic and oleic, which are not formed in the gland, suggests a rapid exchange between glycerides and free fatty acids within the secretory cells. The origin of the milk fat of animals other than ruminants has not been studied in great detail, but rats and rabbits form some fatty acids within the gland from blood glucose [10].

LACTOSE

Lactose is a disaccharide (figure 1). There is a significant arterio-venous difference in glucose across the mammary glands of cows and goats, and many investigators have shown that if glucose, labelled with carbon-14 in all carbon atoms, is injected into a cow or goat both the glucose and galactose of lactose become rapidly and equally labelled. By showing that glucose labelled only in carbon number 1 gave lactose in which the glucose and galactose were also labelled almost only in the same carbon, I was able to prove that this labelling did not come from decomposition products of the injected glucose in the blood. By injecting uniformly labelled glucose into a goat and comparing specific activities of blood glucose and lactose over several hours, O. K. Reiss and I concluded that over half of the lactose came from blood glucose. Similar experiments by Kleiber suggest that about 80 per cent of the lactose in cow's milk is made from blood glucose. There is also evidence that rabbits, guinea-pigs, and rats make lactose from blood glucose [11].

It is clear that cows and goats must use large amounts of glucose for lactose formation, but almost none is absorbed from the gut. Glucose is certainly formed from propionate, but the amounts absorbed from the rumen seem barely sufficient to provide all the lactose of milk. It is conceivable that there might be an inducible pathway by which an overall formation of glucose from acetate occurs, but this does not seem to exist. A very small proportion of radioactive acetate when injected into a cow appears in lactose, and the radioactivity is distributed in glucose as it is after injection into a rat. However, Kleiber has good evidence of formation of glucose from butyrate: a higher proportion of labelled butyrate than of

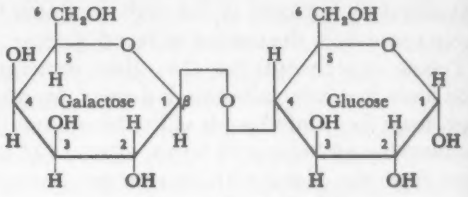

FIGURE 1 – *The structure of lactose* [4].

acetate is converted to lactose, and a lower proportion to milk fat [12].

Cell-free preparations of mammary glands can be made which will convert glucose to lactose, and many experiments have been done to discover the intermediate reactions. A key reaction is certainly the enzymatic conversion of uridine diphosphate glucose to uridine diphosphate galactose, demonstrated in mammary tissue by L. F. Leloir and H. M. Kalckar and their colleagues, following their brilliant experiments on glucose and galactose interconversions in other tissues. J. E. Gander, W. E. Petersen, and P. D. Boyer have good evidence that another fundamental step is the reaction of uridine diphosphate galactose and glucose-1-phosphate to give lactose-1-phosphate. The scheme for lactose formation shown in figure 2 has therefore been suggested.

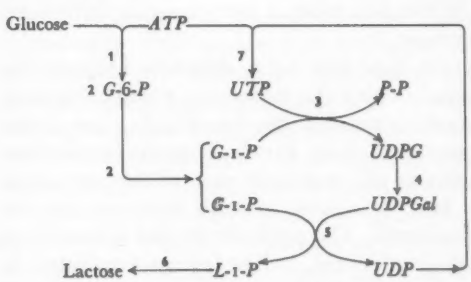

FIGURE 2 – *Suggested pathway for lactose synthesis. ATP, adenosine triphosphate; UDP, uridine diphosphate; UTP, uridine triphosphate; UDPG, uridine diphosphate glucose; UDPGal, uridine diphosphate galactose; P-P, pyrophosphate; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; L-1-P, lactose-1-phosphate* [11].

There are, however, difficulties in this scheme. It can be seen from figure 2 that both the glucose and galactose of lactose are regarded as derived solely from glucose-1-phosphate. Therefore, whenever isotopically labelled lactose is formed by the gland, the glucose and galactose should be identically labelled, provided they do not come from two different pools of glucose-1-phosphate in the secretory cells, which is just possible. If glucose

that has been labelled equally with carbon-14 on every carbon atom is injected into a cow or goat, then the glucose and galactose of lactose do in fact have equal activities; but if the glucose is labelled only in carbon number 1, then the galactose usually has about 70 per cent of the activity of the glucose. This difference can be explained if the glucose molecules that are destined to become galactose are partially oxidized, and recombined to form fewer glucose molecules, by the reactions of the pentose phosphate cycle. However, the difference in activity shows that the scheme in figure 2 is at least partly incorrect. Again, Wood and his colleagues have shown that if radioactive acetate or glycerol is injected into the artery leading to one side of a cow's udder, traces of these compounds are incorporated into lactose, and the galactose has many times the activity of the glucose. He suggests that lactose is principally formed by the reaction of free glucose with uridine diphosphate galactose, the latter also being formed from blood glucose although the intermediates in its formation enter into side reactions. However, no enzyme has been found that catalyses this reaction, and therefore the mechanism of lactose formation is not yet fully understood [11].

CONCLUSION

Finally, what compounds are taken from the blood to provide energy for the gland; what proportion of the total energy does each provide; and how is each compound metabolized in the gland? We cannot yet answer these questions very fully. It seems certain that the glands of ruminants obtain the main part of their energy from substrates different from those of other mammals. It is almost certain that the ruminant gland obtains a large part of its energy by oxidizing blood acetate in the tricarboxylic acid cycle, but the proportion of the total energy which is obtained in this way is not known. There is evidence that the ruminant gland cannot convert glucose to acetylcoenzyme-A and hence oxidize it in the tricarboxylic acid cycle. Glucose is, however, slowly oxidized by slices of the gland, and this probably occurs by the pentose phosphate cycle [13]. The acetate which is absorbed by the gland of a cow during the formation of a litre of milk can be roughly assessed from arterio-venous measurements [14], and only a small fraction of this would be needed to provide energy for the synthesis of the proteins, lactose, and short-chain fatty acids in a litre of milk. However, the amount of carbon evolved as carbon dioxide during the formation of a litre of milk is about the same as the amount of carbon in the acetate absorbed [15]. Of non-ruminants, only the mammary gland of the rat has been studied in detail. Glucose is certainly a major source of energy here, and this can be oxidized via acetylcoenzyme-A and the tricarboxylic acid cycle, but a high proportion is oxidized by the pentose phosphate cycle [13].

Research into the biochemistry of the mammary gland has produced results of great fundamental interest, and this interest will be even greater when we can discover how the chemical reactions of the gland are regulated by the hormones which are carried to it in the blood. The prospect of further discoveries is good, for research into milk secretion is subsidized by medical and agricultural foundations in the wise belief that if we know how the gland works we will be better able to keep it in good order.

REFERENCES

[1] FOLLEY, S. J. *Biol. Rev.*, **24**, 316, 1949.

[2] STEIN, W. H. and MOORE, S. *J. biol. Chem.*, **211**, 915, 1954.

[3] ANNISON, E. F., HILL, K. J., and LEWIS, D. *Biochem. J.*, **66**, 592, 1957.

[4] FOLLEY, S. J. 'The Physiology and Biochemistry of Lactation' (first edition). Oliver and Boyd Ltd, London. 1956.

[5] BARRY, J. M. *Proc. roy. Soc., B*, **149**, 380, 1958.

[6] KLEIBER, M. and BLACK, A. L. 'A Conference on Radioactive Isotopes in Agriculture'. Atomic Energy Commission Report No. TID-7512. U.S. Government Printing Office, Washington. Pp. 395. 1956.

[7] WOOD, H. G., GILLESPIE, R., JOFFE, S., HANSEN, R. G., and HARDENBROOK, H. *J. biol. Chem.*, **233**, 1271, 1958.

[8] HILDITCH, T. P. 'The Chemical Constitution of Natural Fats' (third edition). Chapman and Hall Ltd, London. 1956.

[9] JAMES, A. T., PEETERS, G., and LAURYSSENS, M. *Biochem. J.*, **64**, 726, 1956.

[10] GLASCOCK, R. F. *Proc. roy. Soc., B*, **149**, 402, 1958.

[11] MALPRESS, F. H. *Ibid., B*, **149**, 362, 1958.

[12] ROGERS, T. A. and KLEIBER, M. *Biochem. biophys. Acta*, **22**, 284, 1956.

[13] GLOCK, G. E. and MCLEAN, P. *Proc. roy. Soc., B*, **149**, 354, 1958.

[14] MCCLYMONT, G. L. *Aust. J. agric. Res.*, **2**, 158, 1951.

[15] PETERSEN, W. E. and SHAW, J. C. *J. Dairy Sci.*, **25**, 708, 1942.

Photography of the ocean floor

A. S. LAUGHTON

Although some underwater photographs were successfully taken as long ago as 1893, it is only recently that automatic cameras suitable for photography at depths of up to 3000 fathoms have been developed. This article describes one such type of camera, and discusses the information about the geology and zoology of the sea floor that has been obtained with it.

It was inevitable that, once the photographic method of recording pictures had become established, its use as a means of reproducing underwater scenes and the life of the sea should have been considered. The first serious efforts to take underwater photographs were made by Boutan in 1893, working in the clear waters of the Mediterranean off the coast of France. Although his only successful photographs were made by a diver-operated camera, he nevertheless foresaw the value of an automatic camera which could be lowered to the sea floor in places inaccessible to a diver. His failure in this field was due to the lack of suitable illumination and to the primitive nature of the apparatus then available.

Surprisingly enough, automatic underwater photography was not followed up until nearly fifty years later, although diver-operated cameras taking both still and ciné film had been extensively developed. In 1940, M. Ewing, A. Vine, and J. L. Worzel [1] successfully took photographs of the sea floor with a fully automatic camera. Under the stimulus of wartime needs, photography of the sea floor, especially of wrecks, and of sea-floor conditions for the purpose of sound propagation studies, became an established technique. After the war, it was quickly realized that this was a technique that could be used to investigate the great ocean depths and to observe the abyssal fauna and geology *in situ*. Following the American lead, many laboratories throughout the world have developed their own deep-sea cameras and have used them for a great variety of purposes.

CAMERA DESIGN

Fortunately for underwater photography, the wavelength for which the attenuation of light through water is a minimum lies in the middle of the visual range. In the deep sea we find that water has great clarity owing to the absence of suspended matter and that for ranges up to twenty feet or so no loss of definition of the image occurs. Thus a conventional camera viewing through a plain glass window with suitable illumination will give a good picture. There is, however, one optical problem that arises if high-resolution, wide-angle photographs are required. The path of light from water into air is refracted at an oblique interface, the refraction being a function of the wavelength. Hence, the angular field of view of the lens is reduced and, unless monochromatic light is used, the image off the axis is not sharp. The first effect is not important unless maximum coverage is required. The second effect can be corrected by placing in front of the camera a suitable lens that will introduce an equal amount of chromatic aberration in the opposite sense.

In the shallow water around the coasts and on the continental slope, the turbidity of the water is an important limiting factor. In many places, especially in harbours and river mouths where photography has important practical applications, the visibility is reduced to a foot or less and if photographs are required, a means has to be found of displacing the turbid water in the object field with clear water. This is a difficult practical problem and photography in shallow water is, therefore, of only limited use.

The practical design of a deep-sea camera can best be illustrated with reference to the one designed and built at the National Institute of Oceanography [2]. The camera consists of three basic units, the photographic unit, the flashlight unit, and the acoustic transmitter, or 'pinger' unit. Each of these has its own watertight casing and its own power supplies. The watertight cases are required to withstand external pressures up to 10 000 lb/in^2. The simplest cases for the purpose are cylindrical, and, in order to keep the overall weight to a minimum, an internal diameter of three inches was chosen. The cases are of light alloy, having a wall thickness of $\frac{3}{4}$ inch and being sealed throughout by O-ring seals. The window

for the camera is made of Perspex in the form of a truncated cone 1½ inches thick, in a conical seating.

The photographic unit comprises a lens and shutter, 15 feet of 35 mm film enabling a series of up to 100 pictures to be taken, and a motor and gearbox, driven by torch batteries, to move the film automatically after each exposure. This unit is shown in figure 1. The electronic flash unit is of conventional design working from dry batteries and generates 100-joule flashes. The pinger unit, which is triggered when a photograph is taken, transmits signals through the water to the operator on the surface telling him that the camera has reached the bottom. It is housed in one of the tubes forming the framework. Some such form of indicator is an important part of any equipment which is required to be lowered to the bottom of the deep sea, since it is impossible, in the presence of the additional tension due to up to three miles of wire hanging in the water, and the variations in tension due to the rolling and pitching of the ship, to feel the change in tension on the wire when the camera reaches the bottom. The pinger normally emits signals at a slow rate while the camera is in midwater, but when it touches bottom, the rate is increased.

The camera is triggered by a weight, combined with a small sampler, that is suspended below the camera. When this touches bottom, the flash, synchronized with the shutter opening, is triggered, the pinger signals the surface, and the film is moved to the next frame. The operator can immediately raise the wire several fathoms, so that the camera itself never touches bottom. After a minute or so another picture can be taken and the process repeated. A series of pictures can thus be taken while the ship drifts. In this way a photographic profile can be made of such deep-sea features as seamounts or the edges of abyssal plains. Any standard black and white or colour film can be used, and, if desired, stereoscopic pictures can be taken by using two separate photographic units. The underwater camera is shown in use on R.R.S. *Discovery II* in figure 2.

THE RESULTS OF PHOTOGRAPHIC EXPLORATION

During the past three years many hundreds of photographs of the sea floor, at depths varying from a few fathoms to nearly three thousand fathoms, have been taken by workers at the National Institute of Oceanography. The photographs have an immediate interest in revealing for the first time a part of the earth's crust never before seen by man, but their full scientific value cannot be realized unless they are related to the broader environment in which they are taken. Thus they should be taken in conjunction with a topographic survey of the area and with systematic sampling of the bottom by such means as coring and dredging, so that the details revealed in the photographs can be interpreted in terms of specimens that can be examined at close quarters. This, however, is a counsel of perfection and there is seldom time for all the necessary data to be acquired.

In reviewing the results of the photographic programme to date, it is convenient firstly to consider the photographs in relation to the different regions and features of sea-floor topography, and secondly to illustrate various topics of especial interest that have emerged from a study of the photographs. In some instances, colour photographs are used to illustrate various points, since these give a truer representation of the bottom.

REGIONAL CLASSIFICATION

The nearest and most accessible region of the sea floor is that of the continental shelf, where the water depth seldom exceeds 100 fathoms. The coastal part of the shelf has been frequently photographed by divers and so will not be considered further here. It is difficult to generalize about the nature of the bottom on the shelf since there is as much variety here as there is in the geology of the land. Apart from rocky outcrops and beds of seaweed in the shallower water, a lot of the shelf off the coast of Europe consists of sand, shells, gravel, and mud. The presence of abundant molluscs and other large shells and of coarse gravel is typical of shallow-water shelf deposits. Frequently, one finds the sand patterned by ripple marks, the product of the strong tidal streams and currents around the continents.

As we proceed towards the deep ocean, we come to the continental slope which begins quite suddenly, the depth increasing to 2000 fathoms or more in 20 miles. The slopes are often cut with deep canyons carrying the shallow-water sediments out to the deep sea. Apart from these canyons, the slope is a region of deposition of the fine silt and clay material that has been carried out from the coast. Photographs show a smooth featureless bottom suggesting fairly rapid sedimentation.

In deep water, the bottom of the great ocean basins can be divided physiographically into several regions. Apart from seamounts, ocean trenches, and mountain ranges, the bottom is

more or less completely covered with a blanket of sediment many hundreds of feet thick. Some areas reflect the buried topography in an undulating bottom, and others have an extraordinary flatness associated with sedimentation by turbidity currents. These are currents of water containing sediment in turbulent suspensions that can flow along gentle gradients on the sea floor for many hundreds of miles. At close quarters, however, both the abyssal plains and the undulating bottom are very similar. The ooze is soft enough for extensive reworking by bottom-living organisms which leave tracks, burrows, mounds, and other evidence of their existence. In some cases there are indications of the originator of these features, such as the impression of a five-armed star on the top of many of the small humps, but more often one is left to guess when and how they were made. Occasionally, one is lucky enough to see a sea cucumber or a brittle star crawling along the bottom, or some tube worms or a sponge sticking out of the ooze, but the density of life in abyssal depths is very low. Typical photographs of this region are shown in figures 3, 4, and 12.

The great mid-Atlantic ridge divides the Atlantic Ocean into eastern and western basins. This vast chain of mountains has an obscure origin and much work has been done to elucidate its structure but without any conclusive results. Photography has shown that its rocky peaks are areas of extremely low sedimentation, and have been subject to recent and violent upheaval. Figure 5 shows steep slopes and freshly cleaved rocks resembling the screes found on land. Other photographs have shown basins of sediment covered with patches of shingle-like material that may in fact be colonies of a deep-sea shellfish. On the whole the rocky peaks appear poor in sessile fauna, a fact consistent with the idea of a geologically recent disturbance.

By far the most interesting and varied collection of sea-bottom photographs has been taken on the volcanic seamounts that rise out of the deep basins. In general the greater the depth, the less dense is the faunal population. The attraction of a seamount to the abyssal fauna is doubtless partly related to its geological formation. There are a great many exposed rocks and boulders in varying states of fracture; there are sands and oozes in great variety. These offer to the fauna a variety of footholds and, to fishes, shelter in which to hide from their foes. Possibly nutrients are concentrated in the water in these regions by local upwelling due to obstruction of the water flow and by the solution of minerals from the rocks themselves. Examples are shown of the continuous type of submarine lava flow, of the bedrock, of broken boulders and shingle, and of the current-swept sand with ripple marks. Often it appears that the fauna of one seamount differs from that of another and that where in one place for instance one finds predominantly crinoids, in another there may be sponges. However, this differentiation may only be the result of inadequate sampling techniques and until more pictures are taken it cannot be confirmed. Photographs taken on seamounts are illustrated in figures 6–11 and 13–16.

Photographs were taken of one bank of especial interest off Cape Finisterre. Galicia Bank, which is divided from the continental shelf by a depth of 1500 fathoms, has a structure that may be related more nearly to that of the continent than to that of the ocean floor. Rocks dredged from it are of two distinct types; limestones and a collection of dark metamorphic rocks. The photographs strongly suggest that the limestone is locally derived, whereas the metamorphic rocks are erratic and have possibly been carried by icebergs from the north (figure 17).

SPECIAL PROBLEMS

The geological interpretation of rocks seen in bottom photographs is extremely difficult unless hand specimens can be examined. However, some very interesting features of deep-sea geology have been revealed. In particular, photographs have indicated the distribution and the environment of the rocks. In the Pacific Ocean, millions of tons of potential manganese ore have been found scattered on the deep-sea bed in the form of manganese nodules. The exposed surface of nearly all rocks in deep water slowly acquires a coating of manganese dioxide and this can be used to indicate which way up a piece of rock lay. In figure 5 the white patches on the broken boulders are probably places where a manganese coat is lacking and one can infer from this that they have been disturbed quite recently from the position in which they have lain for many thousands of years. The cauliflower texture seen on the rock on the left of figure 16 is due to a similar manganese crust.

Underwater lava flows have been found photographically in a region where a dredge has been unable to pick up any loose material from the bottom. In figure 6, photographed from the American research vessel *Vema*, the roundness of the rocks is similar to that of the pillow lavas found on continental rocks and believed to have been solidified

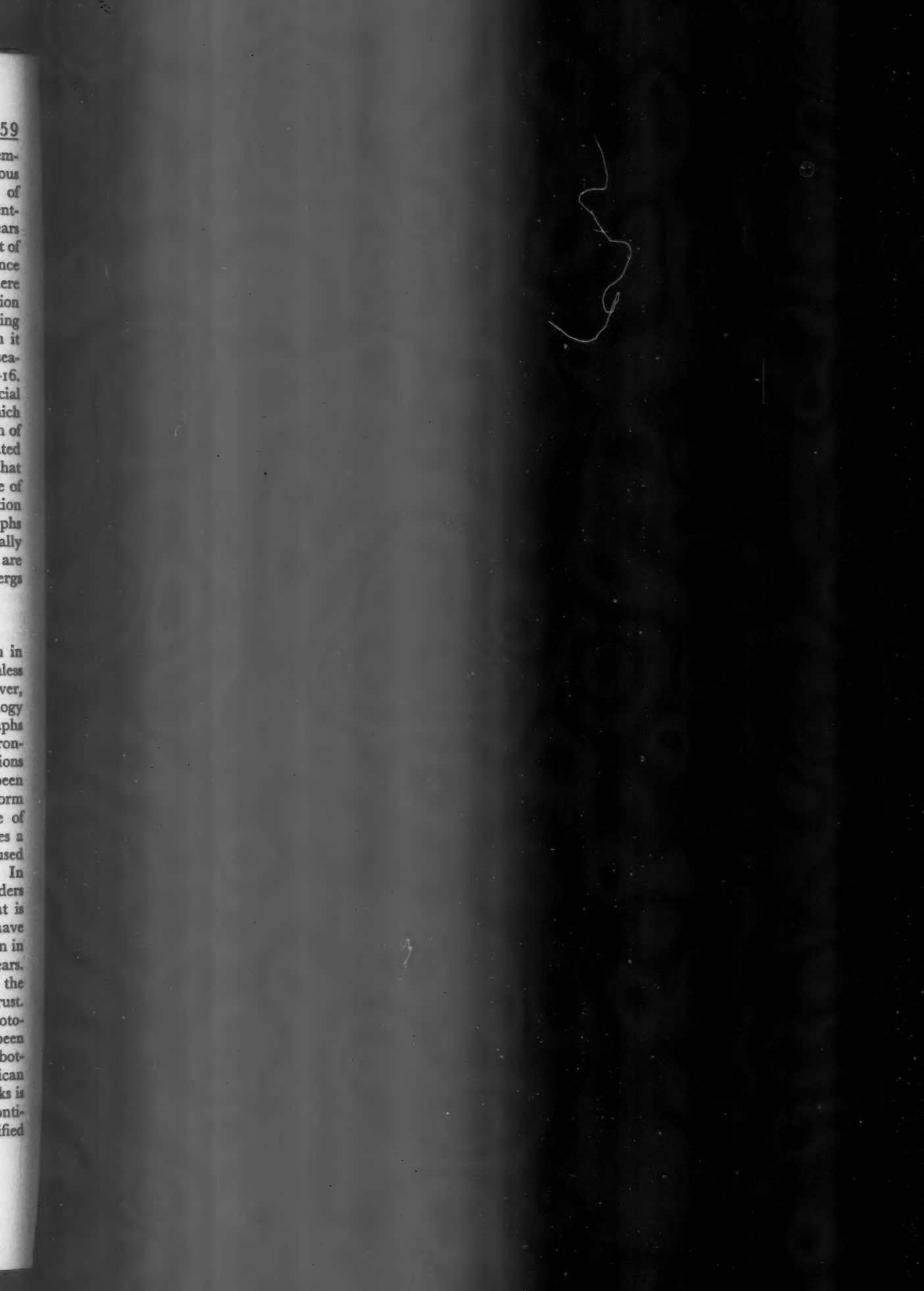

FIGURE 1 (*left*) – *Photographic unit, showing batteries, motor, gear-box, film drive mechanism, and lens. (This model has no shutter.)*

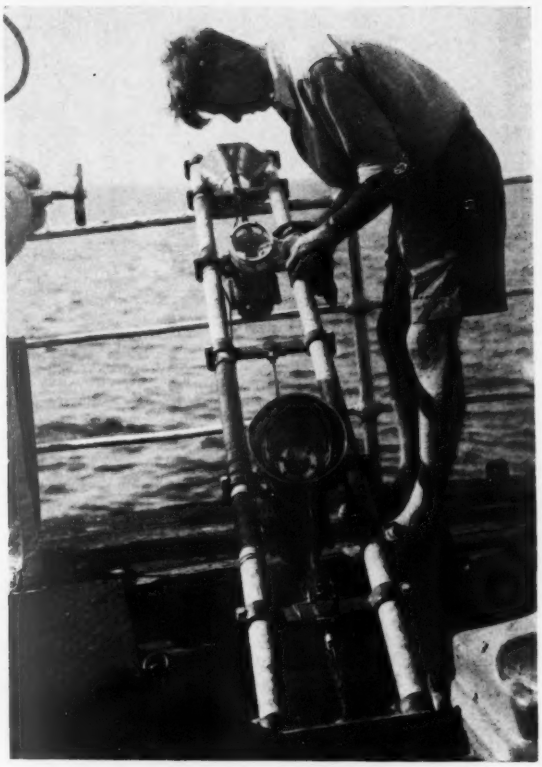

FIGURE 2 (*right*) – *Deep-sea camera in operation. Units are: acoustic transmitter (at the top), photographic unit, flash unit, bottoming switch, trigger weight, and sampler.*

URE 3 – *Typical view of deep-ocean basin sediments in 2550 ms, showing the extensive activity of bottom fauna in creating ng and mounds.*

FIGURE 4 – *A four-inch-wide track in the ooze of a deep basin (2890 fathoms) perhaps made by an animal travelling on the surface and scooping the mud into the central ridge while feeding.*

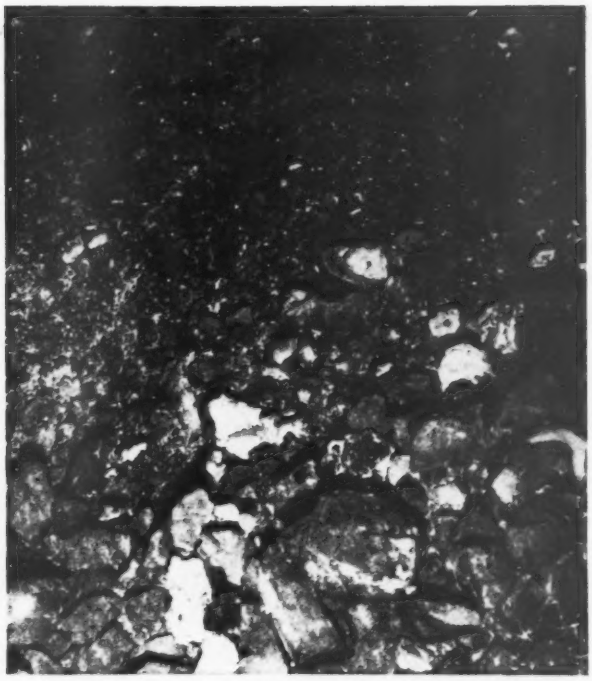

FIGURE 5 – *Scree slopes on a peak on the mid-Atlantic ridge at a depth of 800 fathoms, showing absence of sediment and some signs of recent disturbance.*

FIGURE 6 – *Oblique view of an underwater lava flow on the si of a volcanic seamount at 720 fathoms.*

FIGURE 7 – *Rock and calcareous sand on a seamount at 1580 fathoms. Feathery comatulid crinoids are seen attached to branching corals.*

FIGURE 8 – *Gorgonians, pennatulids, sponges, corals, and sea urch on the top of a seamount at 75 fathoms, showing the increased fau population in shallow water.*

FIGURE 9 – *Outcrop of bedrock on a volcanic seamount at* 800 *fathoms, showing a zeid fish, three varieties of gorgonians, anemones, and some spicular debris deposited by current action. The central gorgonian is more than* 5 *ft high.*

FIGURE 10 – *Ripple marks, scour, and sand-drifts in a calcareous sand on a seamount at* 1710 *fathoms.*

FIGURE 11 – *A rare holothurian* (Peniagone), 8 *inches long, showing feet, branching tentacles around its mouth for feeding, and its 'sail' in a collapsed condition (extreme left). Depth* 1560 *fathoms.*

FIGURE 12 – *Soft globigerina ooze on an abyssal plain at 2900 fathoms. A pale-blue sea lily sits on a burrow (12 inches in diameter). Note the radial feeding tracks of a buried animal.*

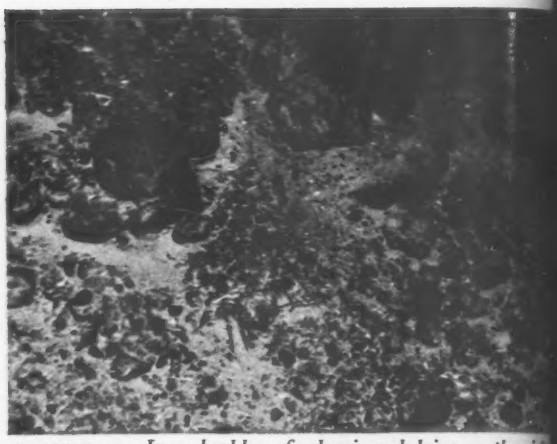

FIGURE 13 – *Loose boulders of volcanic rock lying on the side seamount at 1730 fathoms. Two five-armed comatulid crinoid attached to the rocks and miscellaneous organic debris is sca between.*

FIGURE 14 – *Boulders and cobbles lying in loose sediment on the side of a seamount in 1810 fathoms. Of special interest is the gravel-size material lying in the lower right-half of the picture which, on close examination, has the appearance of mollusc beds.*

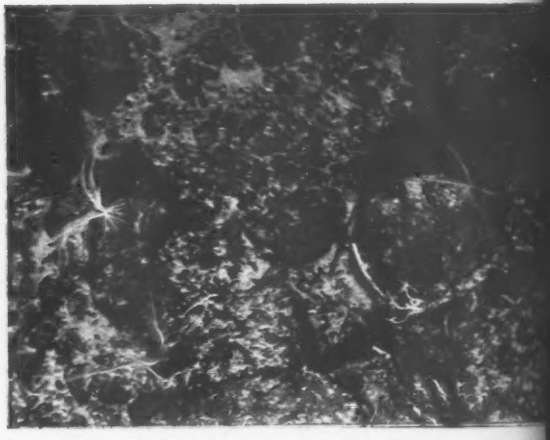

FIGURE 15 – *Five- and ten-armed crinoids attached to bou on the side of a seamount at 1840 fathoms. A pink brittle sta a red deep-sea prawn (in the shadow of a rock, lower right be seen.*

FIGURE 16 – *Crinoids and a brittle star attached to broken coral at 1890 fathoms. The surface of the left-hand rock has the typical 'cauliflower' appearance associated with the growth of a manganese crust.*

FIGURE 17 – *On Galicia Bank at 350 fathoms. The pale r (probably limestones) are locally derived, whereas the dark r have been carried from elsewhere.*

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under water. Once again the nodular manganese coating is plainly visible.

The ripple-marks found in the deep sea are of great interest to sedimentary geologists. At one time it was thought that the deep sea was a region of very small currents and that the existence of ripple-marks in a sedimentary rock indicated deposition in shallow water. We now have direct evidence of ripple-marks being formed in deep water together with scour and sand drifts around obstacles in the sand. Figure 10 shows these features in globigerina sand at a depth of 1700 fathoms.

Almost every photograph taken in the deep ocean shows evidence of some kind of life existing there. In most cases it is impossible to give a specific name to the animals found, since the systematic classification has been based on detail that is often not visible in a single picture. However, the pictures do show the animals in their normal habitat and in undamaged condition, whereas many of the dredged specimens have suffered damage in collecting, and distortion in the resultant pickling and storing in formalin. Figure 11 shows an unusual holothurian that was previously known only from extensively damaged specimens collected by H.M.S. *Challenger* in 1875.

One of the most fascinating puzzles posed by the pictures is the identification of the tracks and burrows found over the wide areas of the soft bottom. One must appreciate that with the sediment accumulating at the slow rate of only a centimetre or less in a thousand years, a track once made will be visible for a very long time. Thus the density of tracks reflects the activity of several thousand years. Some of the tracks are made by animals on the surface and some by animals beneath it. Figure 4 shows the broadest tracks found (about four inches across) and is representative of many such tracks found beneath the Atlantic. A similar track, but with much more pronounced transverse marks was photographed by Zenkevitch from the Russian research ship *Vityaz*, in the Pacific. Although the central part of the track is raised, it must have been formed by an animal on the surface because of the regularity of marking. A possible interpretation is that the track was made by a mud-feeding animal which scoops the mud into its mouth with some form of arms or tentacles, producing transverse marks and a ridge of ooze, the central depression being made by a trailing tail. Possible animals to fit this theory are decapod crustaceans and holothurians of the type shown in figure 11.

Other interesting tracks are the sinuous and the spiral forms. These appear to have been made by worms burrowing just below the surface and stop suddenly when the worm burrows downwards. The shapes of these tracks provide interesting evidence of the way in which an animal can search an area most economically and systematically.

Many other interesting problems arise out of these photographs concerning the relationship between fauna and their environment. Photography will doubtless be used more and more in the future to study deep-sea marine ecology.

FUTURE USE OF UNDERWATER PHOTOGRAPHY

It is clear from the above discussion that deep-sea photography has its place in any investigation that includes the sea bottom. A photograph is second-best to direct observation, but until bathyscaphes become more common, direct observation is not possible. It may be advantageous, in sampling the bottom, to attach a camera to the corer or dredge to estimate the sampling technique more efficiently. Certainly, if it becomes a practicable proposition to drill into the solid rock of the sea bed, photographic control would be most desirable.

The deep-sea camera has been used as a tool in a number of ways other than those described. Fish, squid, and the deep scattering layer have been photographed in midwater using suitable triggering devices and the camera has been incorporated as the recording method for the measurement of currents on the sea floor. Doubtless many other uses will be found in the future.

ACKNOWLEDGMENTS

I am grateful to the Director of the Lamont Geological Observatory, New York, for permission to publish the photographs shown in figures 6 and 8. Figure 2 is published by permission of the British Petroleum Company.

REFERENCES

[1] Ewing, M., Vine, A., and Worzel, J. L. *J. opt. Soc. Amer.*, 36, 307, 1946.
[2] Laughton, A. S. *Deep-Sea Res.*, 4, 120, 1957.

The Darbys of Coalbrookdale

A. RAISTRICK

This year is the 250th anniversary of the first successful smelting of iron with coke, by Abraham Darby, an event of outstanding importance in the history of technology. Coalbrookdale, the scene of this achievement, has since remained an important centre of the iron industry. There the cylinders of many of the early steam engines were cast, Abraham Darby III built the world's first iron bridge, the first iron rails were made, and the first high-pressure steam engine was constructed for Trevithick. Later, Coalbrookdale played an important part in the development of cast iron as a constructional material for a wide variety of purposes.

The introduction of the blast furnace from the Continent at or just before the beginning of the sixteenth century marked the opening of a new phase in the history of ironmaking in Britain, for not only was the plant more elaborate but the process of obtaining malleable iron was different. The old bloomery process [1] gave directly an iron of the quality required for forging. It worked at a temperature too low for fusion, and in consequence harmful impurities such as sulphur and phosphorus did not enter the iron as they did when blast furnaces were used. Pig iron made in blast furnaces had a high carbon content, and this had to be reduced, in a separate operation, before the iron was suitable for most of its uses. This reduction was effected in fineries, in which the cast iron was melted and stirred: it was then consolidated by hammering, being reheated at intervals in the chafery. These additional operations called for extra labour and fuel, but in the long run these disadvantages were outweighed by the greater productivity of the blast furnace. Productivity became a major factor as the demand for iron increased, but the primary reason for the adoption of the blast furnace in Britain seems to have been military. Many charcoal-burning blast furnaces were established: by 1600 nearly a hundred were in operation, more than sixty of them in the Weald of Sussex area, where gun founding was the mainstay of the industry [2].

The seventeenth century saw the exploitation of the iron ores in the Forest of Dean and of coalfield ores, with a corresponding migration of ironmaking into south Yorkshire and the Severn valley, and a concentration of forges and smithies to manufacture iron goods, in the Midlands [3]. The coalfield ores contained a substantial amount of phosphorus (0·15–0·42 per cent) and so made a pig iron that was brittle when cold ('cold-short'), but the Forest of Dean ores were almost free of phosphorus (0·07 per cent). When pig iron from these two sources was mixed in the forge a very good wrought iron resulted. This circumstance, with the advantage of easy transport along the Stour and Severn, eventually established the Midlands and Welsh Borders as great iron-making and iron-using areas, with Bristol as the natural market and port for their products.

This concentration of furnaces and forges in the Severn area created problems, however. The competition for charcoal became severe, and in consequence its price increased threefold. Attempts to make working more economical by increasing the size of the blast furnace were frustrated by the fact that charcoal has too little mechanical strength to support a column of ore more than about 25 ft in height: when the charcoal was crushed by the load on it, the blast was choked. A cheaper and stronger fuel was therefore a prime necessity for the advance of the industry, and it is not surprising that many experiments were made to use coal in place of charcoal in blast furnaces. Dud Dudley claimed to have achieved success in 1619, but his claims are not acceptable [4]. Many others experimented, and some, such as Grandison, Sturtevant, Addison, and Rovenson, obtained patents; none, however, achieved real success. One great difficulty was the presence of sulphur in the coal, since this adversely affects the metal. As a result, attention was directed to the possibility of using coke, for the process of coking eliminates the sulphur. This was already known to maltsters, who, for quite other reasons, had to avoid contaminating their product with sulphur. Moreover, coke was beginning to be used at the start of the eighteenth century in copper-smelting and lead-refining, so that it was not an altogether strange product in the metal industry. Coke used in malt kilns near Derby was made by firing open heaps of

coal covered with dust: this gave a yield of approximately 33 per cent.

Such, in very brief outline, was the background to the successful introduction of iron-smelting with coke by Abraham Darby (1678–1717) in 1709. By both birth and early training he was well qualified for this achievement. He was born near Dudley, Worcestershire; his father and grandfather were locksmiths, and he thus had many opportunities of observing the smith's use of iron. As a young man he was apprenticed to a maltmill maker, and so must have learned of the use of coke by maltsters and its methods of preparation. In 1699 he set up as a maltmill maker in Bristol, his choice of this city doubtless being decided in part by the fact that the Quaker community, to which he belonged, was strongly represented there. Bristol was a centre for copper and lead smelting, and in 1702 he formed the Bristol Brass Wire Company, with other Quakers as his partners. One of them was Nehemiah Champion, whose family was for several generations intimately connected with the brass and copper industry and with the iron trade. In copper-smelting a charge of two-thirds coke and one-third coal was then being used in a furnace resembling the steel cementation furnace, and Darby must have become very familiar with this practice before he turned his attention to iron.

With his brass-making enterprise well established at Bristol, Darby in 1708 formed the Bristol Iron Company, where he put into operation a patent he had been granted in 1707 for casting iron 'bellied pots' (or cauldrons) in dry sand. He was soon seeking a place to make his own iron, and eventually leased the Old Furnace in Coalbrookdale: this stood near the Severn in an area where coal and ironstone had been mined for many years. One of the local coals, from the Clod seam, was a good coking coal, low in sulphur. Darby probably knew of its properties from his earlier experience, and it was doubtless this that led to his choosing this particular coal for his first experiments in smelting iron. The furnace was leased from September 1708, and by January 1709 it had been repaired and blown in. By 25th January 1709 'a parsell of new pigs 4 tons 5 cwt 2 qr 0 lbs at £6. 10. 0. per ton' was sent to Bristol. This was a major landmark in the history of iron-making, for it was the first commercial iron to be smelted with coke, an achievement which marked the birth of modern iron technology.

Darby used the iron from his furnace to cast pots and other hollow ware. One very important feature of his work was the thinness of the castings, which allowed his cast-iron pots to compete successfully with the heavier brassware then widely used. Coke gave a higher hearth temperature in the furnace than was reached with charcoal and in consequence more silicon in the pig iron; this factor, together with the fact that the phosphorus content of the ore was high, resulted in a very liquid melt, and consequently relatively low risk of blowholes in the castings.

At that time a charcoal furnace was usually blown for 25–30 weeks in the year, the rest of the year being needed to accumulate sufficient charcoal: one furnace could absorb the available charcoal from a considerable area. By contrast, a coke furnace could be blown for 48 weeks in the year, and a coalfield could support a number of furnaces without any interruption of fuel supply. The output of a charcoal furnace was about 15 tons of iron a week, and although an early coke furnace would make only about 5 tons a week this was soon improved on; moreover, the better fuel supply and the longer blast allowed a number of coke furnaces to work together and provide whatever increased output was desired.

The first Abraham Darby died in 1717, and the works were continued by his son-in-law Richard Ford (1689–1745), who had been employed by Abraham Darby as his clerk and assistant in the works. In 1728 Abraham Darby II (1711–63) was taken into the works and ten years later became a partner in the business. Of the children of his second marriage two sons, Samuel and Abraham III (1750–89), and two daughters, Mary and Sarah, all took some part in the business. Under Ford the casting of engine cylinders, pumps, and pipework was begun at the Old Furnace, and he continued casting pots and other articles at the New Furnace, built in 1715. Cast-iron cylinders were so much cheaper than brass, and easier to make in large sizes, that they soon became the only ones used in the atmospheric (steam) engine, of which the first satisfactory example was built by Newcomen at Dudley Castle, Worcestershire, in 1712. By 1758 the Company had cast more than 100 cylinders for Newcomen engines, including some of very large size. For example, one cast for an engine installed at the Walker Colliery, Newcastle upon Tyne, had a diameter of 6 ft and a length of 10½ ft: it weighed 6½ tons. The immense technological significance of the Newcomen engine needs no emphasis: the beam-engine conquered flood water in mines and revitalized the mining industry.

Attempts to improve the furnace performance were dominated by the need for a stronger blast, and one that would be available throughout the year. The water-wheel then used was not satisfactory, as it could not be worked when the stream was low. In 1733 horse-driven pumps were used to return for re-use water that had already turned the water-wheel that worked the bellows. In 1742 Abraham Darby II applied a steam engine to this task and secured a great improvement in production.

The market for cast-iron goods was not very extensive, and the pig iron produced in the coke furnace was difficult to refine into good wrought iron because of its high phosphorus content; consequently the sale of pig iron outside Coalbrookdale was not very important. As late as 1760 only seventeen coke-burning blast furnaces were in operation in Britain. From 1748 onwards, however, Abraham Darby II gave his attention to the problem of producing from the blast furnace an iron suitable for the forges. He ultimately solved it chiefly by selecting the most suitable ores available, those known locally as Black, Ball, and Penny ironstones, containing about 0·1 per cent phosphorus. In 1753 he built two furnaces at Horsehay and two more at Ketley: these four furnaces each had an output of 15 tons a week, raised in the next ten years to 25 tons each. A substantial part of the pig iron so produced was sold to the Midland ironmasters, such as Knight and Foley, for conversion to bar iron; only then was the industrial success of coke smelting established. Coke-fired furnaces were built by Wilkinson in Staffordshire; by the ironmasters of South Wales, Yorkshire, and Scotland; and by the end of the century by those of France, Silesia, and Prussia. Abraham Darby I was the pioneer of coke-smelted iron for the foundry; Abraham Darby II was the originator of coke-smelted iron for the forge and rolling mill.

The Dale Company, as it was then known, developed its own forges and produced bars and sections both for use in its own manufactures and for sale, and it built a large mill to make boiler plates. By 1785 four more furnaces had been built and there were sixteen steam engines serving the works and furnaces, nine forges, rolling mills, and furnaces for the casting shops. The capital of the Company was more than £100 000 [7].

By the provision of cheap cylinders of large diameter for steam engines Coalbrookdale made a major contribution to the development of the mining of both coal and metals, and this interest continued until the present century. Two immediate problems created by the cylinder-casting business were the handling and transport of massive castings of iron about the works and to the wharf, and the making of a large melt, weighing several tons, for a single casting. The first was met by an extensive system of tram roads, some twenty miles in all, between pits and furnaces and about the works; this was established in 1748 and improved in 1767 by the substitution of the world's first cast-iron rails for old wooden ones.

A widely known monument to the works' capacity to cast and handle large masses of iron is the famous Iron Bridge across the Severn, half a mile below Coalbrookdale, the first iron bridge in the world (figure 2). This was designed, cast, and erected in 1779 by Abraham Darby III and was the forerunner of a long series of bridges, the making of which was later transferred to the Horsehay Works; this production has been continued and extended by the present Horsehay Company since 1886. For the Iron Bridge castings, the Old Furnace in Coalbrookdale was reconstructed and in this form it is still in the works (figure 3), and was displayed by the Coalbrookdale Company (Allied Ironfounders Ltd) to mark the 250th year of their existence. The use of structural cast iron had developed both in iron-framed buildings by 1796 [8] and in a variety of architectural castings, columns, railings, and smaller items.

The versatility of the Company and their readiness to experiment encouraged engineers to come to them with unusual demands. In 1756, 1759, and again in 1763 James Brindley was in the works supervising the making and testing of a steam engine and of a new type of boiler of his own invention. A local engineer, Joseph Hornblower, for many years from 1735 onwards, had castings for Cornish mine engines made at Coalbrookdale. The family connection was maintained for at least three generations: in 1781, for example, Jonathan Hornblower sent his son to discuss his idea for a compound engine, which was successfully built and used in the works, and was eventually brought into general production. Adam Heslop, brought up in the works, invented an engine with two cylinders, one 'hot' or operative, using the steam under pressure, the second one 'cold', taking the used steam and condensing it, so acting as an atmospheric engine. It was in fact one of the first compound steam engines and was granted a patent in 1790. The action was smooth, and was preferred to the Boulton and Watt for use on many of the Coalbrookdale mines and plant.

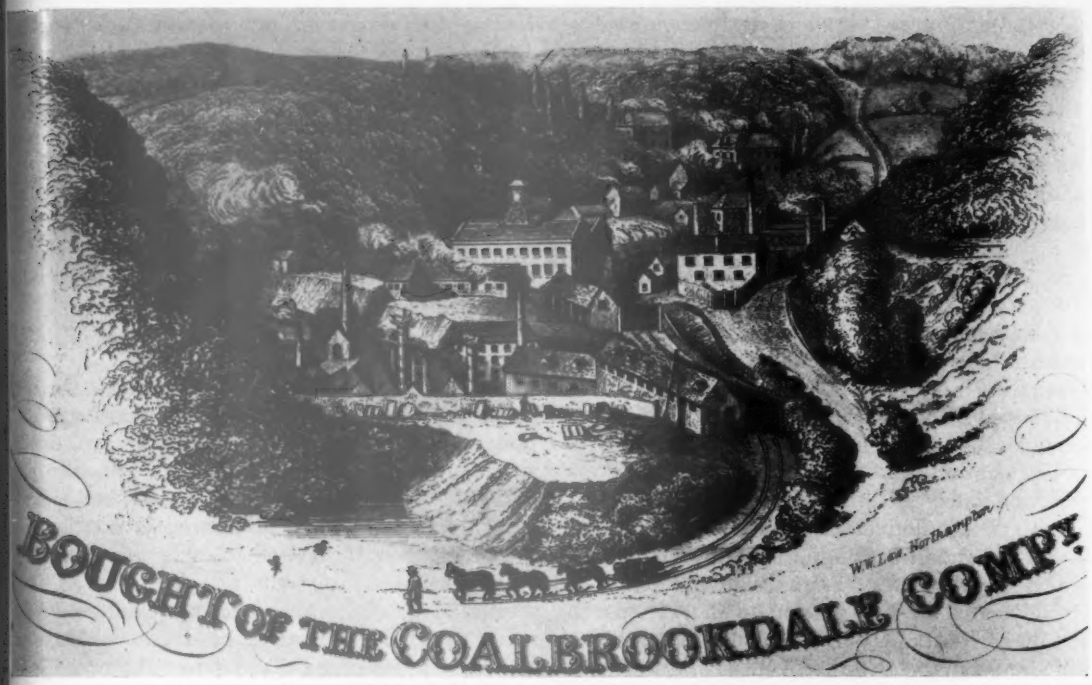


FIGURE 1 – *Engraved billhead showing comprehensive view of Coalbrookdale works,* 1843.

URE 2 – *Trade token of* 1792, *showing the Iron Bridge at rookdale.* (× 2½)

FIGURE 3 – *Coke-fired blast furnace at which the Iron Bridge castings were made.*

Later, Sadler came to Coalbrookdale to have his ideas for an improved tandem steam engine translated into practice. Several engines were made to his plan and, like Heslop's, were used in the mines; later Sadler had them produced for the naval dockyards at Plymouth.

In 1790, the Company began to make boiler plates of large size by rolling; these in due course made possible the construction of boilers working at high pressures. In 1796 Richard Trevithick, who, like his father, had had experience of Coalbrookdale engines in Cornwall, visited the works. In 1802 he came again, this time to have a high-pressure boiler made and tested, and his first rail locomotive built. Coalbrookdale already had experience in this field, for as early as 1787 William Reynolds had made experiments in steam locomotion within the works, but a fatal accident to a workman had caused them to be abandoned. The Trevithick locomotive, the first in the world to run on rails, is almost certainly represented in a well-known drawing of a 'Tram Engine' which was wrongly attributed by Mason to the Penydaren locomotive [10]. A boiler and pumping engine built for Trevithick worked with great success at 145 lb/in^2 pressure—a pressure far beyond any previously attained at that date, when engines still normally used steam at little above atmospheric pressure. On 22nd August 1802 Trevithick wrote to his friend David Giddy, later President of the Royal Society, 'The Dale Co. have begun a carriage at their own cost for the realroads and is forceing it with all expedition' [9]. Several locomotives were built during the nineteenth century for use about the works. From these early experiments stemmed the general use of high-pressure steam and the modern steam locomotive.

In the first decade of the nineteenth century a process of rationalization resulted in the plant and operations being divided into several well-defined units. Output from the forges had already reached 1200 tons a month—very high by the standards of the day—and the employees numbered over 2000.

During these years of reorganization there had been a general depression in the iron trade, and in 1816 the works at Ketley were given up. However, by 1830 there was a recovery, and the Company started on another phase of development. Under the guidance of Francis Darby (1783–1850), the son of Abraham Darby III, there was a great development of artistic and architectural castings and of the manufacture of gates and railings. In 1851 the Company exhibited in the Great Exhibition and received a Council Medal. At that time it had between three and four thousand work-people, and output was 2000 tons a week: it was the largest ironworks in the world (figure 1).

The nineteenth century was remarkable for the development of cast iron as a constructional material, in which use the Company had set a striking pioneer example with the Iron Bridge, and this is fully illustrated in its products. The amazing variety of articles offered in cast iron—the catalogues listed thousands of different articles—is today difficult to appreciate. There seemed to be no limit to what cast iron could be made to do: its use varied between the finest of art castings—filigree fruit plates or the reproduction of da Vinci's 'Last Supper'—and such massive work as the Hyde Park Gates and the Albert Edward railway bridge over the Severn between Coalbrookdale and Buildwas. Solid-fuel appliances were a notable feature of production, and manufacture of these has continued up to the present day.

From 1827 Francis Darby shared the management of the various concerns with his brother Richard and with Abraham IV and Alfred, the grandsons of Samuel Darby. Abraham IV retired from Coalbrookdale in 1849 to take an active interest in the Ebbw Vale steelworks. Francis died in 1850 and Alfred in 1851, and thus the long line of Darby managers came to an end. Alfred's son, Alfred II (1850–1925), however, became a director of the Coalbrookdale Company and was the last Darby to be connected in any way with the works. As a family, their contribution to the iron industry was remarkable.

REFERENCES

[1] SCHUBERT, H. R. 'History of the British Iron and Steel Industry'. Routledge and Kegan Paul, London. 1957.

[2] STRAKER, E. 'Wealden Iron'. Bell, London. 1931.

[3] JOHNSON, B. J. C. *Econ. Hist. Rev.*, 2nd Ser., IV, 1951–52.

[4] MOTT, R. A. 'Dudley and the Early Coal-Iron Industry'. *Trans. Newcomen Soc.*, **15**, 17, 1934–35.

[5] HOUGHTON, J. 'Husbandry and Trade Improved'. London. 1727.

[6] MOTT, R. A. *Gas World*, **50**, 7, 1957.

[7] Letter from Richard Reynolds to Lord Gower, 28th March 1785, quoted in RAISTRICK, A. 'Dynasty of Ironfounders. The Darbys and Coalbrookdale', p. 97. Longmans Green, London. 1953.

[8] HOBBS, J. L. *Shropshire Mag.*, No. 3, pp. 43–44, 1950, and letter Morley Tonkins, 6th September 1950, quoted in RAISTRICK, A., *loc. cit.*

[9] RAISTRICK, A. *loc. cit.*, pp. 161–68, 1953.

[10] FORWARD, E. A. *Engineer*, **193**, 266, 1952.

Spectrophotometric technique for studies of respiratory enzymes in living material

H. LUNDEGÅRDH

The enzymes that are involved in cell respiration have, in general, colours that change during oxidation and reduction. They can therefore be studied in the living cell by absorption spectrophotometry. It has been necessary to develop special instruments for these investigations, and this article describes the instruments and some of the results achieved with them.

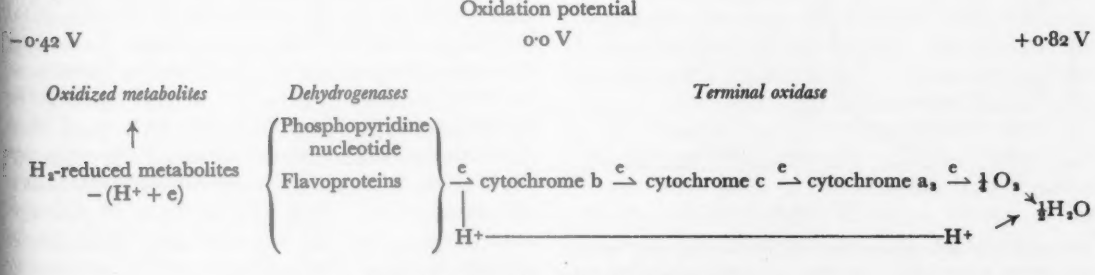
The spectrophotometric technique developed during the last decade has been greatly influenced by studies of respiratory enzymes. In addition to the universal key position of respiratory enzymes in cell metabolism, an obvious reason for selecting them for experimental study is the fact that most of them are pigments which change colour on reduction or oxidation. Spectrophotometry offers the possibility of studying their operation in actually living cells and tissues.

The respiratory chain, a scheme of which is given below, is composed of dehydrogenases, which remove hydrogen from metabolites, and oxidizing enzymes, which, through a series of electron transfers, perform the end oxidation. The large amount of energy developed in the total process is trapped in high-energy phosphate at a number of points in the chain, and only a little disappears as heat.

Each enzyme in the respiratory chain is reduced by one compound and oxidized by another [2], and the corresponding transference of electrons may be followed by observation of spectral changes. The oxidized cytochromes show colours from greenish (cytochrome a_3) through light red (cytochrome c) to deeper red (cytochrome b) and are characterized by sharp absorption bands, the γ-bands or Soret bands, in the blue and blue-violet (see table 1) and have only broad indistinct bands in the green and red. During reduction the γ-bands move 10–20 mμ towards longer wavelengths, and new bands (α- and β-bands) appear in the green (cytochromes of c- and b-type) or the red (cytochromes of a-type). Only cytochrome c has been isolated in a pure state, but the cytochromes b and $a+a_3$ also yield comparatively good preparations, which make possible measurements of molar extinction, and of the shape and position of the absorption bands.

Of the dehydrogenases, the coenzymes I and II (di- and tri-phosphopyridine nucleotide) are colourless, but a characteristic ultra-violet band at 340 mμ appears upon reduction. The flavoproteins are colourless in the reduced state but turn more or less yellowish on oxidation, owing to the appearance of a broad band at about 450–470 mμ and a second band in the near ultra-violet.

Because the respiratory pigments are present as individual chemical compounds in the living protoplasm, it is theoretically possible to perform an optical resolution of the total system and to follow the oxidation and reduction of the single enzymes, provided that the true absorption curves of these are known. Modern spectrophotometric methods are here distinctly superior to visual spectroscopy.



Scheme of the respiratory chain of enzymes

TABLE I

Principal absorption bands of enzymes in the respiratory chain. The molar extinction values (mol^{-1} l^{-1} cm^{-1}) *vary between ca.* 6×10^3 *and ca.* 3×10^4. *Wavelength values in* mμ.

	α-bands		β-bands		γ-bands	
	Oxidized	Reduced	Oxidized	Reduced	Oxidized	Reduced
Cytochrome a_3	None	598	None	None	420	444
Cytochrome a	None	604	None	None	—	452
Cytochrome b	Indistinct[1]	562–564	Indistinct	530	416	430
Cytochrome c	Indistinct	550	Indistinct	520	406	416
Cytochrome c_1	Indistinct	554	Indistinct	522	408	418
	Oxidized		Reduced			
Flavoprotein	450–470		None			
Phosphopyridine-nucleotide ..	None		340			

[1] 'Indistinct' means a broad elevation of the spectrum, the height and shape of which are important in the calculation of the difference spectrum. The broad indistinct α-β-band of oxidized cytochrome c is nearly as high as the β-band of the reduced enzyme (see figure 1).

The instruments mentioned in this article were constructed primarily for a detailed analysis of the enzyme system which carries on aerobic respiration in higher plants and yeast. The respiratory systems in micro-organisms and in plant and animal cells are built along similar lines, but there are differences of detail. The new technique has shown that cytochromes c, c_1, b, a_3 (cytochrome oxidase), and a, all known to exist in animal cells, also participate in the respiration of wheat roots. Plants invariably contain quantities of another haemin enzyme, peroxidase, which shows certain additional oxidizing qualities. Because the technique permits accurate measurements of the extinction at exactly reproducible wavelengths, it has been possible to observe the operation of the individual enzymes in living cells and tissues, as well as in homogenates and in particulate fractions of the protoplasm.

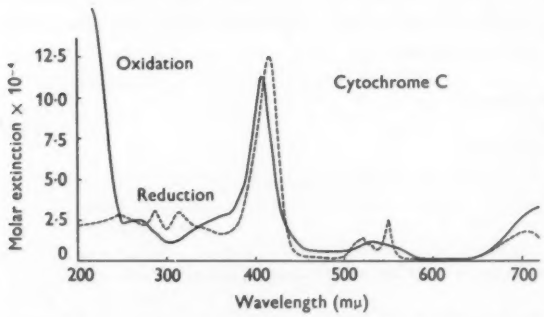


FIGURE 1 – *Absorption spectrum of oxidized and reduced cytochrome c, recorded in intervals of* 1 mμ *with the instrument shown in figure* 3.

These studies of the kinetics of the steady states reveal characteristic differences in the patterns of reduction and reoxidation of the single enzymes which are difficult to explain in terms of free reactants. The co-operating enzymes apparently react as multi-molecular groups which are linked together in definite patterns. The respiratory unit may be looked upon as one of the fundamental building stones of living protoplasm. According to scattered reports in the literature, a number of accessory substances or factors serve as junctions or switchboards for controlling the stream of electrons through the system. Some of the enzymes and factors are sensitive to specific inhibitors, as a consequence of which certain lines of electron transference may be switched off while others remain active. These studies reveal interesting properties of the different enzymes, such as the

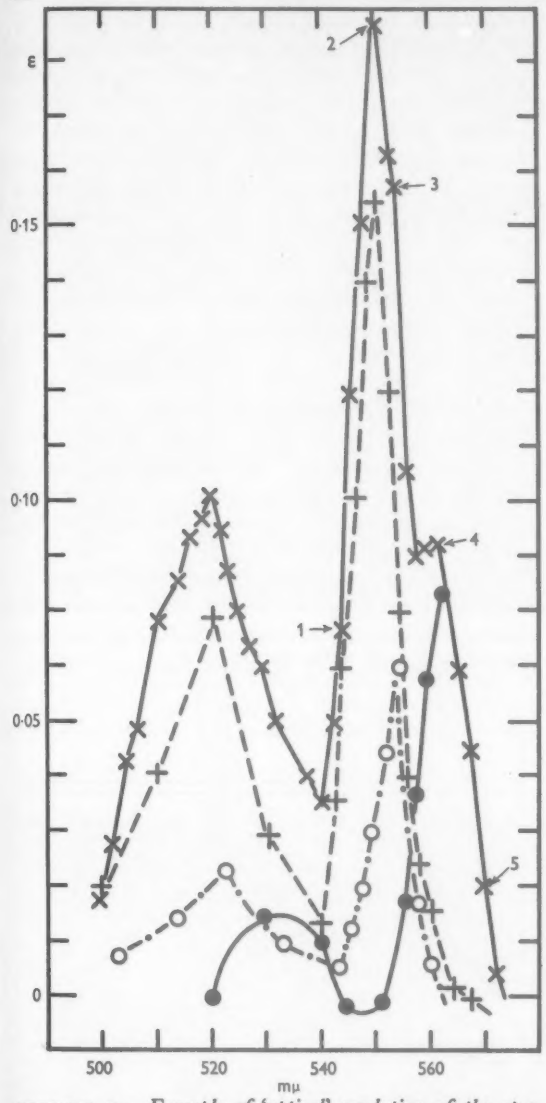


FIGURE 2 – *Example of 'optical' resolution of the spectrum.* ×——×, *observed spectrum of a* 15 *per cent suspension of washed yeast gassed with* N_2 (*extinction of reduced yeast minus extinction of oxidized yeast*). +------+, *spectrum of cytochrome c, calculated from the difference* 2–1. ●——●, *spectrum of cytochrome b, calculated from* 4–5. o-·-·-·-o, *spectrum of cytochrome* c_1, *calculated from the difference at* 554 mμ (3) *between the observed spectrum and the calculated spectrum of cytochrome c.*

fact that, of the closely allied cytochromes a_3 and a, only the former acts as an oxidase, and the fact that cytochrome b in plants may act as a b-shunt to oxygen if a_3 is inhibited. Considerable differences in function also exist between the twin enzymes c and c_1 and the frequently co-existing members of the cytochrome b family.

Recording spectrophotometers of high sensitivity facilitate synchronous observations of the activity of the respiratory enzymes and of specific physiological processes, such as the accumulation of salts in plant cells. This last process is one of the fundamentals of the mineral nutrition of plants, and is to some extent also responsible for the turgescence of parenchymas. In its electronic activity the respiratory unit acts as a miniature electric machine, and its activity is in fact related to the concentration of electrolytes in the surroundings, that is, to the conductivity in the external circuit. This non-specific salt effect can be demonstrated by direct spectrophotometric observation of changes in the steady states of the cytochromes when the tissue is supplied with salts. Only plants turn this general effect into an active salt pump. The spectrophotometric observations show a participation of both cytochrome oxidase and cytochrome b in the salt effect. Cytochrome b and, in general, enzymes of the protohaematin type, are more dominant in plants than in animal tissues.

TECHNIQUES

Visual spectroscopy served excellently during the pioneer work of MacMunn and Keilin on cytochromes [1], but the use of a simple spectroscope is of necessity restricted mainly to a statement of the position of visible bands. The spectroscopist is frequently confused by spectra of mixtures of pigments, because the combination of adjacent bands of two separate compounds may result in a misleading shift in the position of the peaks. Only exact measurements of the bands in terms of molar extinction permit a reliable resolution of combined or mixed absorption spectra. An example of the method of making such measurements and calculations is given in figure 2. The general procedure is a successive subtraction of calculated bands of known compounds from the observed mixed spectrum.

Measurements of the extinction at various wavelengths may be made by means of a conventional spectrograph, using photometric evaluation of the spectrogram. This method is, however, tedious and has so far been very little used in biochemical work. Spectrophotometry started with the construction of photo-electric cells by Elster and Geitel. Such cells, in combination with sensitive millivoltmeters, yielded excellent results in the hands of O. Warburg and his group [3], and served as a useful tool in their work on the *Atmungsferment*. For work with living material the

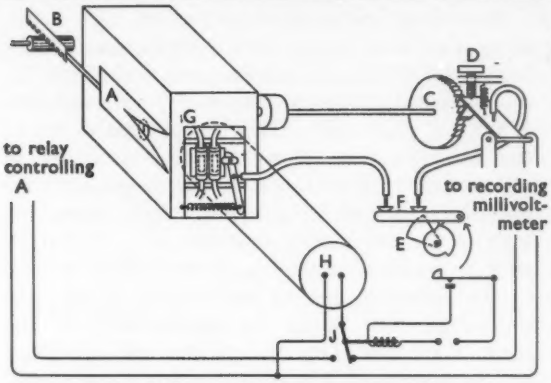


FIGURE 3 – *Scheme of the automatic grating spectrophotometer: A, diaphragm; B, gear connected to a balancing motor and a potentiometer for control of A; C, a wavelength timer set by the screw D and operated from a cam E which is driven by a synchronous motor. The lever F co-ordinates the operation of the wavelength timer and the sliding object-holder G. H, photomultiplier tube; J, device for alternate switching over of the photocurrent from recorder to balancing motor.*

sensitivity of the cells had to be still further augmented, and an instrument was also constructed by means of which flame photometry was developed as a method for studies in mineral nutrition [5]. Later on the introduction of photomultiplier tubes still further encouraged the construction of sensitive spectrophotometers [4].

In the last decade a number of manufacturers have placed convenient spectrophotometers on the market. Instruments made commercially, however, are as a rule adapted for general laboratory work, and a spectroscopist attacking special problems must still depend upon his own constructions.

A spectrophotometer capable of detecting the presence and activity of respiratory pigments in living material must show a high sensitivity, corresponding to an amplification of the primary current from the photocathode by a factor of 10^8–10^{10}. Photomultiplier tubes provided with a quartz window and 11–13 dynodes (total voltage 1500–2000 V) are as a rule sufficient for accurate recording of spectrograms from 15–25 mm thick layers of plant tissues (roots or storage tissue) or suspensions of yeast (5–15 per cent) and other micro-organisms in tubes of 20 mm diameter. The anode current may be still further amplified by an extra amplifier, but this is needed only under exceptional circumstances. Tungsten lamps (for the visible spectrum) and hydrogen lamps (for the ultraviolet) may be recommended as light sources.

Only recording instruments are of real use for studies of living material and, because of the lability of the steady states of oxidation-reduction, instruments that work rapidly are to be preferred. For recording extensive spectra, the instrument must be provided with a device for elimination of the varying spectral sensitivity of the photocathode. Different methods may be used for turning the varying intensity of the reference beam into a constant intensity over the whole measured spectral range, but they all divide the ingoing light into two beams, one for reference and the other for the sample. Adjustment of the reference beam may be made by regulation of the anode current, for example by means of a shunt. This method, however, introduces a possible error, because at high sensitivities the inevitable dark current of the photocell varies considerably with a change in the total resistance of the circuit. This error may be avoided by the use of an alternative method, the automatic adjustment of the intensity of the ingoing light by means of a movable diaphragm (see figure 3).

A disadvantage of instruments which draw a continuous spectrum on a strip of paper is the tedious and not very precise procedure of checking the wavelengths. The operator has to divide up

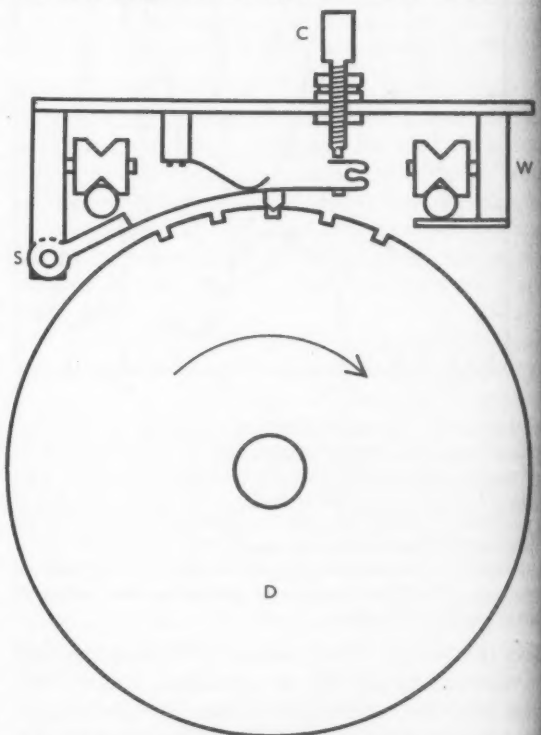
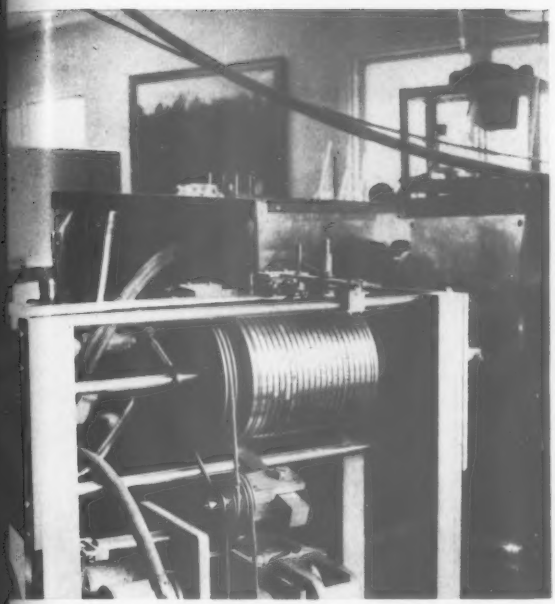


FIGURE 4 – *Details of the contact device which regulates the shift of wavelength positions of the quartz monochromator (cf. figure 5). D, drum; W, sliding carriage with a contact spring S and an adjustable pin C.*



URE 5 – *Wavelength drum of the quartz spectrophotometer (for ils see figure 4). An electromagnetic clutch stops the movement of rum each time the contact pin plunges into a notch. In this instru- notches are cut at intervals of 2 mμ. Below the drum are the elec- agnetic valves for the pneumatically regulated sliding object-holder.*

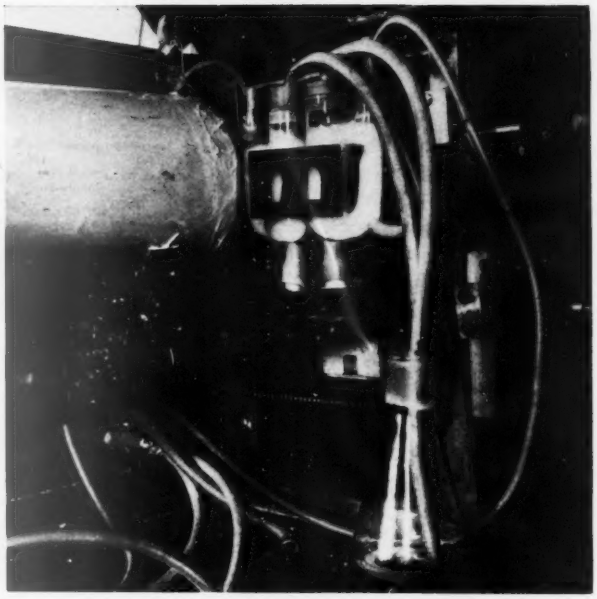
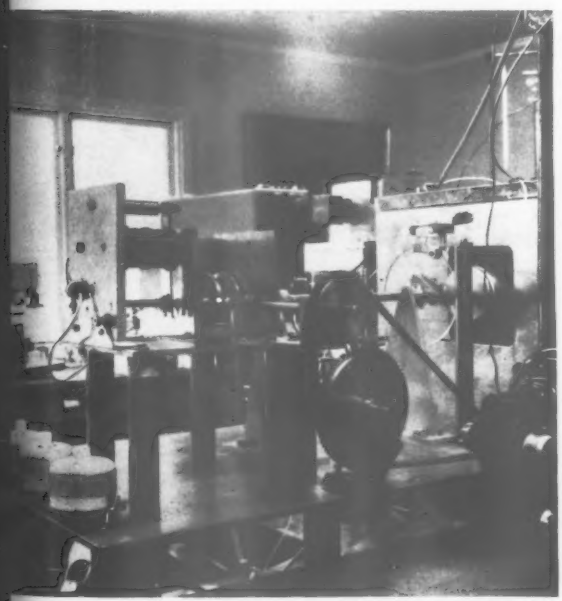


FIGURE 6 – *Details of the sliding object-holder (c.f. figure 3) which in this case carries two circulation vessels containing 15 per cent yeast, one aerated by O_2, the other by N_2. To the left is the photomultiplier.*



URE 7 – *View of the scanning device from the opposite side, wing the double bevel-gear and the transmission. To the left, the romagnet which reverses the movement of the bevel-gear. To right, the device for suppressing the oscillographic image at ain wavelengths.*

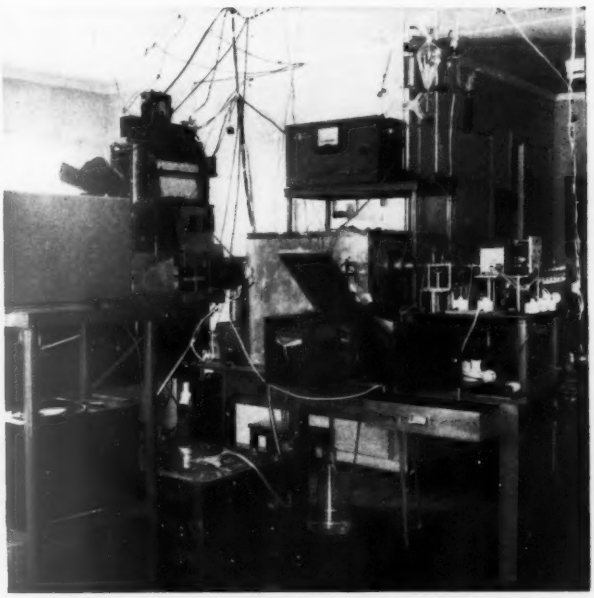


FIGURE 8 – *Grating monochromator mounted with the interchangeable devices for continuous or scanning recording. To the left, the oscilloscope with a camera, which is provided with gears for constant feeding of the film. Below, a shielded carriage containing the 2000-volt battery for the photomultiplier. Above, an extra amplifier, and arrangements for continuous flow of solutions or gases through the receiver.*

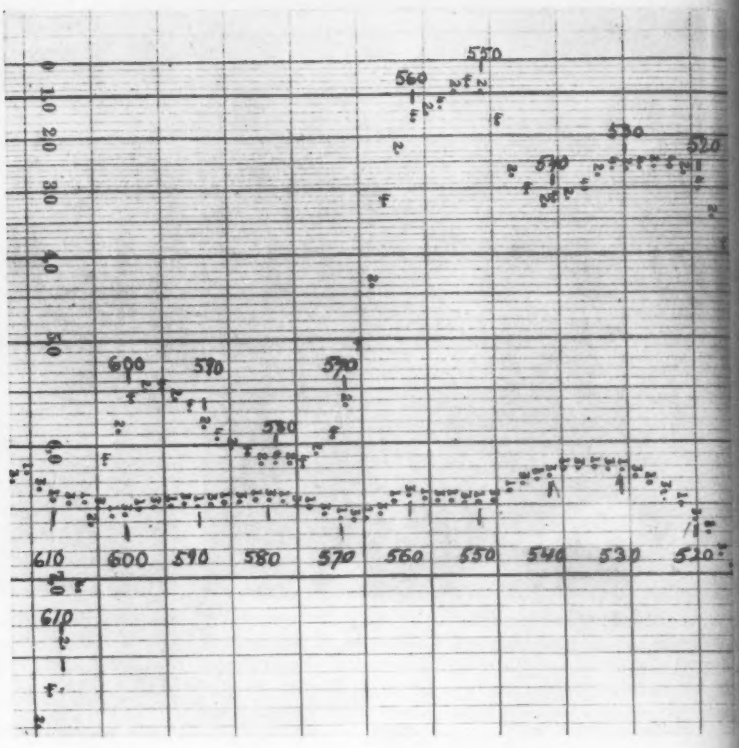


FIGURE 9 – *Record of a 4 mm thick cake of baker's yeast with eight layers of filter paper as the reference. Upper curve = sample; lower curve = reference (ε = 1–2, 3–4). The reference beam has been approximately equalized by means of the cam device.*

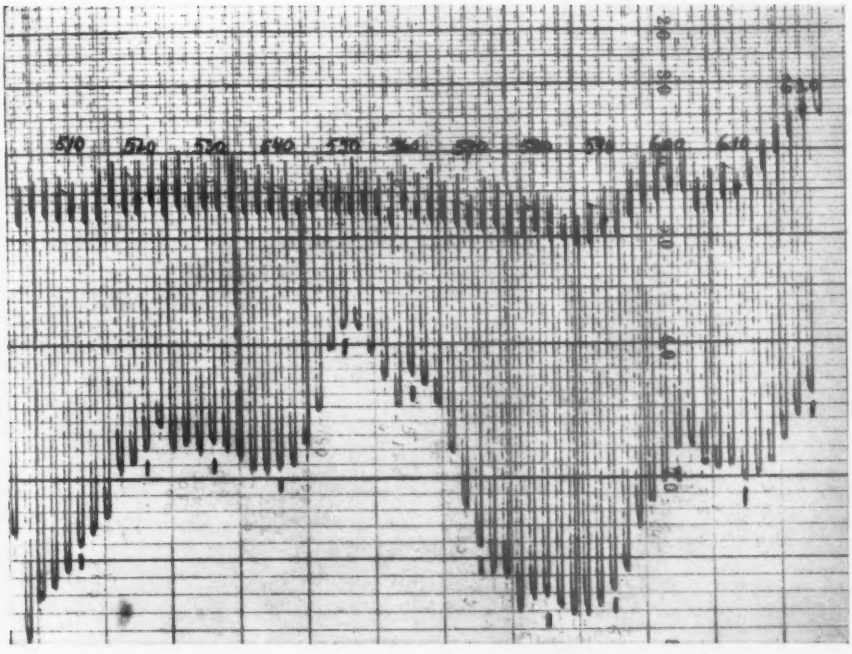


FIGURE 10 – *Record of suspension of 15 per cent washed yeast, aerated with air (= reference: dots) or pure nitrogen (= sample: vertical dashes). Extinction in the difference spectrum is the difference between the points marked on the upper curve and the corresponding ones on the lower curve. The reference beam has been approximately equalized by means of the automatic device shown in figure 3.*

the spectral curve by means of ruler and pencil, and the reproducibility of the measurements is not very good. Another disadvantage of instruments drawing a continuous curve is the hidden influence of the inertia of the millivoltmeter. Each meter has its minimum period of deflection, and a continuously moving pen will be completely balanced only when the recorder is moving comparatively slowly. Only oscillographic recording removes this source of error.

One possible way of avoiding these difficulties is to use instruments which divide the absorption curve into a series of single, individually recorded steps. In these instruments the wavelength drum is automatically moved in equal intervals of one or more millimicrons and full equilibrium of the voltmeter is established between steps. The construction of the instruments shown in figures 3 and 5 ensures that the wavelength positions of the drum are exactly reproducible, a circumstance that improves still further the accuracy of the measurements.

Of the two instruments constructed in the author's laboratory, one is provided with a quartz-prism monochromator, the other with a grating monochromator. Owing to the exponential decrease of the distance between the wavelength positions on the drum, which is characteristic of prism instruments, the stepwise movement requires a fairly complicated machinery, the details of which are shown in figures 4 and 5. The drum is provided with engraved furrows at each, or alternatively at every other, mμ-value. Each time a contact pin (see figure 4) drops into a groove the drum stops, always at exactly the same position. The timer steering the rhythm of the instrument starts the movement again and the next groove stops it, and so on.

A grating monochromator offers the advantage of equal divisions on the wavelength drum. Instead of the complicated machinery needed for the operation of the prism monochromator, the movement of the wavelength screw may be controlled simply by a toothed wheel. The intermittent shifting of the wavelengths in this instrument is performed mechanically by means of a flexible steel cable connected to a rotating cam device, shown in figure 3. The periods may be set at one or more seconds, and the mμ-values of the intervals may be changed freely.

The dotted curves drawn by one of these instruments (see figures 9 and 10) yield very reliable readings of the extinction and of differences of extinction between two wavelengths. The author's experience of spectral analysis of living material has taught him that curves recorded in 2 mμ intervals satisfy all demands for exact measurements of band heights. Intervals of 1 mμ may be chosen for special purposes, such as determination of the positions of peak values, but it is more important to know exactly the extinction values in 2 mμ intervals than to know the position of a peak, the value of which always varies somewhat according to the choice of slit width in relation to the dispersion of the instrument. A strictly quantitative comparison between two spectrograms is of course possible only if both are recorded at identical slit widths.

The classical way of measuring extinction ($\varepsilon = \log \{I_0/I\}$) implies separate measurements of intensities of the reference beam, I_0, and of the beam passing through the sample, I. It was mentioned above that some modern instruments substitute the recording of I_0 by automatic elimination of the sensitivity curve of the photocell. The operator has in this case to trust the guarantee of the manufacturer; he is unable himself to check the values of I_0. The intermittent operation of the author's spectrophotometers offers the possibility of checking the value of I_0 at each recorded wavelength. This brings another advantage, namely that it is not necessary to eliminate variations in the sensitivity of the photocell completely, a fact which makes possible a high speed of recording. If each value of I_0 is recorded, an approximate adjustment is quite sufficient, because the logarithmic difference between I_0 and I is measured for each individual wavelength. The readings and calculations are considerably facilitated if the paper is logarithmically divided (see figures 9 and 10).

The instruments are provided with a mechanical or pneumatic device for switching between reference and sample, as shown in figures 3 and 6. The adjustment of the intensity of the reference beam is performed just before the recording of I_0.

Because living material is more or less turbid and much light is lost by scattering, the reference beam must pass through a neutral substance of similar turbidity and scattering qualities to the sample. Layers of filter paper (such as 8–11 layers of Munktell No. 3), cotton wool, etc, have proved useful for this purpose. It is a familiar experience that the direct spectrum of the sample may be distorted as compared with the spectrum of clear solutions. Distortion is avoided if two oxidation-reduction states are recorded by difference spectra as I_0 and I respectively. The increasing reduction

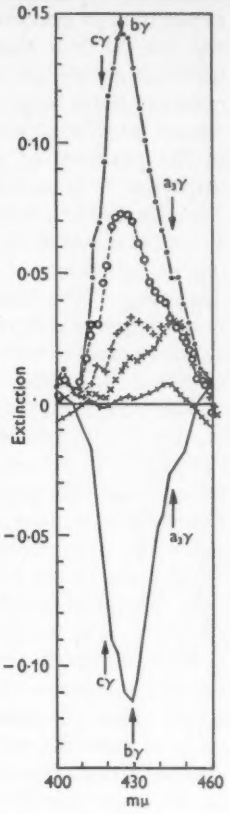


FIGURE 11 – *Example of resolution of the spectrum by means of intermittently recorded spectrograms as reduction proceeds. Material: a 14 mm thick bundle of wheat roots (drainage by aerated water, 0·2 cm³ per second). The spectrum of the aerated sample serves as reference. Time from shifting over the medium to an oxygen-free solution of succinate (positive curves): ++++ 1 min., x-x-x- 4 min, +--+-- 7 min., o----o 13 min., ·——· 32 min.; ——reoxidation 1 min. after switching over to aerated solution (negative curve). It is clearly seen that reduction starts with the cytochrome oxidase ($a_3\gamma$ at 444 mμ), later followed by cytochrome b ($b\gamma$ at 430 mμ). Wheat roots contain only little cytochrome c ($c\gamma$ is visible at 416–418 mμ). On complete reduction (32 minutes from start), only a large fused band is seen, on which bands of cytochromes a_3 and c may be traced from small elevations.*

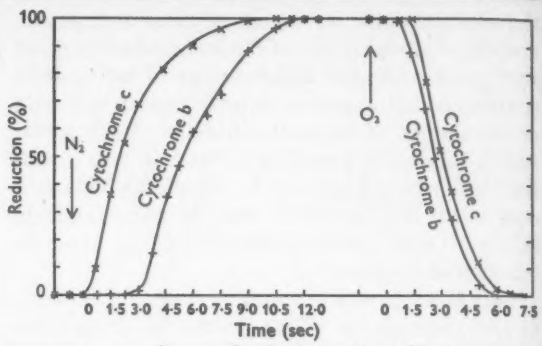


FIGURE 12 – *Course of reduction and reoxidation of cytochromes c and b recorded at 20° C with the oscillographic scanning device, spectra recorded in intervals of 0·3 sec. The extinctions are measured from the differences 550–546 mμ (for cytochrome c) and 562–570 mμ (for cytochrome b), for the sake of comparison, however, calculated in percentages.*

of bakers' yeast may thus be studied if one tube with oxygenated yeast suspension serves as the reference for a similar tube containing a suspension fed with nitrogen. The percentage increase of reduced cytochromes, pyridine nucleotides, and flavoprotein may be calculated from the difference spectrum according to the procedure mentioned above. For measuring the extinction of the single cytochromes, wavelength intervals are chosen in regions where the bands of the other cytochromes show a minimum of interference (see table 1 and figure 2). The quantity of interfering 'accessory' cytochromes may be approximately calculated from the deviations in the bands of adjacent well-known cytochromes: thus cytochrome c_1 can be estimated from the deviation of the extinction at 554 mμ from the calculated cytochrome c value. In calculations of the actual concentrations of enzymes in living material, due attention must be paid to the influence of the turbidity. Correction factors may be computed from direct measurements of the extinction caused by the addition of known quantities of pure enzymes to the turbid samples [6].

A valuable complement to the method of quantitative optical resolution of mixed spectrograms is the segregation of steady states brought about by changes in the degree of oxygenation or by the influence of specific inhibitors. Cytochrome b may thus be 'optically purified' by addition of antimycin A, which inhibits the reoxidation of this cytochrome. Observation of the characteristic pattern of reduction is also of great value. Cytochromes a_3 and c start the reduction, cytochrome b and flavoprotein follow (figures 11 and 13). The time sequence is commonly reversed at reoxidation. The characteristic rhythm during increasing reduction of the respiratory system may be interpreted as a reflection of the spatial organization. The individual enzymes are apparently reacting with one another in multimolecular groups which are, at certain points, joined by accessory substances or 'factors', some of which may be related to vitamins.

Because of the complexity of the total respiratory chain, a complete analysis of its properties requires the recording of fairly complete spectrograms. The speed of the recorders is also a factor of importance. Details of the rapidly changing steady states may be surveyed if an instrument is used which records the region 520–620 mμ (covering α- and β-bands of most cytochromes) in 100–200 seconds. Rapid changes in the steady states of single enzymes may be followed by continuous recording of the difference between two wavelengths [7]. For similar purposes, direct recording of the peaks may also be used [6].

Large portions of the spectrum may be extremely rapidly recorded by means of an oscillograph. In the scanning spectrophotometer the spectrum is flickered on to a photocell attached to

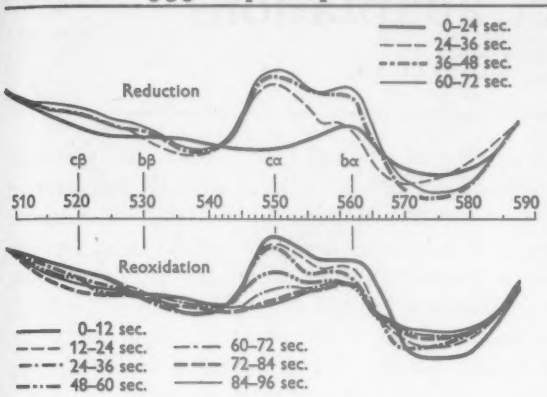


FIGURE 13 – *Slow scanning (period 12 seconds) of the processes of reduction and reoxidation of a sample of 15 per cent washed yeast, enclosed in a circulation vessel and aerated by nitrogen (for reduction) or air (for oxidation) at 5° C. Records made by a rapid Speedomax G recording millivoltmeter. The slower start of reduction of cytochrome b, as compared with that of cytochrome c, is clearly shown, as is also the gradual shift of extinction above or below the isosbestic line (for comparison see the changes of the spectrum of isolated cytochrome c in figure 11). At reoxidation the timing is reversed, cytochrome b going ahead of cytochrome c.*

an oscillograph. Series of spectrograms may thus be recorded in fractions of a second. Because of the noise which always to some extent disturbs measurements at these extremely high amplifications of the photocurrent, it is not advisable to go below about one-tenth of a second, however. According to the author's experience, intervals of $\frac{1}{3}$–$\frac{1}{2}$ second (see figure 12) are sufficient for accurate recording of changes in the steady states of micro-organisms upon removal or addition of oxygen and useful observations may even be made at intervals of 10–12 seconds if the processes are slowed down by cooling the containing vessel. A simple and convenient instrument for scanning work is shown in figure 7. An electrically operated double bevel-gear moves the wavelength screw to and fro. The length of spectrum recorded may be chosen at will, but a practical limit is set by the shape of the sensitivity curve of the photocell. For still more rapid work, scanning of the spectrum may be effected by a rotating or oscillating mirror which is placed between the collimator lens and the outgoing slit of the monochromator.

Examples of the usefulness of the scanning spectrophotometer are shown in figures 12 and 13. The pattern of reduction and reoxidation of the respiratory system of living material is difficult to explain unless it is assumed that the enzymes are operating in multimolecular units which are reacting with one another at spatially fixed points or surfaces. The method is thus a useful tool for tracing the existence of a spatial organization of the enzyme systems. Spectrophotometry may also reveal the influence of spatial organization on the molecular stoichiometry. Observations pointing to such influences, which may cause shifts in band positions, have been made in connection with studies of coenzyme I [6] and of photosynthetic pigments [8]. The variation of the band positions among haemin enzymes may also depend upon a variation of, or, alternatively, in the mode of connection to, the apoenzymes. Only three or four different haemins are known as prosthetic groups of respiratory enzymes, whereas the number of corresponding enzymes is considerably larger. The elucidation of these problems is, however, largely dependent on future progress in preparative and organic chemistry.

REFERENCES

[1] KEILIN, D. *Proc. roy. Soc., B*, 98, 312, 1925.
KEILIN, D. and HARTREE, E. F. *Ibid., B*, 122, 298, 1937.

[2] JAMES, W. O. 'Plant respiration'. Clarendon Press, Oxford. 1953.
Idem. Advanc. Enzymol., 18, 281, 1957.

[3] WARBURG, O. *Angew. Chem.*, 45, 1, 1932; 'Schwermetalle als Wirkungsgruppen der Fermente'. Berlin, 1946.

[4] FRENCH, C. *Carnegie Inst. Wash., Yearb.*, 53, 182, 1954.
DUYSENS, L. *Science*, 120, 393, 1954.
WITT, H. *Naturwissenschaften*, 3, 72, 1955.

[5] LUNDEGÅRDH, H. 'Die quantitative Spektralanalyse der Elemente', Vols. I and II. Jena, 1929–34. 'Die Nährstoffaufnahme der Pflanze'. Jena. 1932. 'Leaf Analysis.' London, 1951.

[6] *Idem. Ark. Kemi*, 5, 97, 1952; 7, 451, 1954.
Idem. Nature, Lond., 171, 521, 1953.
Idem. Biochim. Biophys. Acta, 20, 469, 1956; 25, 1, 1957; 27, 355, 653, 1958.

[7] MILLIKAN, G. A. *Proc. roy. Soc., B*, 123, 218, 1937.
CHANCE, B. *Science.*, 120, 767, 1954.

[8] JACOBS, E. E., HELT, A. S., KROMHOUT, R., and RABINOWITCH, E. *Arch. Biochem.*, 72, 495, 1957.
LATIMER, P. *Science*, 127, 29, 1958.
SMITH, H. C., KUPKE, D. W., LOEFFLER, Y. F., RENITEZ, A., ARNE, I., and GIESE, T. 'Research in Photosynthesis', p. 464. 1957.

Brazilian insect adaptations

H. B. D. KETTLEWELL

Eastern Brazil gave Charles Darwin his first experience of tropical forest and its inhabitants and contributed to the formulation of his theory of evolution. The author of this article has lately visited the same region to study insect adaptations there in the light of modern genetical and evolutionary theory. He suggests that a very rich flora may account for the large insect fauna, and that long-standing environmental stability may have permitted the preservation of frequent extreme specialization. Increased biological pressure resulting from the existence of an almost infinite number of biological niches, together with a high predation rate, may, the author concludes, be a further reason for Brazil's remarkable insect fauna.

If we wish to meet some of the most extreme forms of specialization in life it is probably true to say firstly, that they can be found among insects; secondly, that these will be tropical insects; and thirdly, that they are likely to occur in those equatorial regions of the world that have been subjected to no extreme climatic changes in the recent past. On these assumptions, conditions in Brazil are exceptionally favourable. It was here that in 1832 Charles Darwin, at the age of twenty-two, was for the first time in his life confronted with tropical forest and its inhabitants. It is clear from his 'Journal of Researches' [1] and his 'Origin of Species' [2], though less so from his 'Diary of the Beagle' [3], that the insects of Brazil made a great impression on him: the Lampyridid phosphorescent beetle; the Ageroniid butterfly which 'clicked' in flight; the Army ants and their behaviour; the Pepsis wasps. Nevertheless, he wrote in the 'Journal' the following revealing sentence: 'The large and brilliantly coloured Lepidoptera bespeak the zone they inhabit, far more plainly than any other race of animals. I allude to only the butterflies; for the moths, contrary to what might have been expected from the rankness of the vegetation, certainly appeared in much fewer numbers than in our own temperate region.' This is a most poignant sentence, because on the one hand it suggests Darwin's quite natural inability to assess, at that time, the significance of conspicuous butterfly patterns. On the other, it admits the efficiency of the camouflage of the multitudinous cryptic (camouflaged) moths which so successfully hid from him by day: modern collecting methods have made an appreciation of them much easier.

It is indeed unfourtnate that Darwin had initially to meet what were probably the most complex inter-relationships to be found amongst insects in the world. His main interests in Brazil were, however, centred on orders other than the Insecta. At a later date, much of what he had observed was interpreted scientifically, in agreement with his own views, by Alfred Russell Wallace [4] who had been collecting on the Amazon, which, incidentally, Darwin never visited. Wallace, in contrast to Darwin, arrived at his concept of Natural Selection and the evolution of one species from another, largely as the result of studying insects.

Last year the centenary of the joint Darwin-Wallace paper [5] was celebrated and I was fortunate enough to be sent out to visit the same places in Brazil as Darwin went to in 1832. My assignment was to attempt to evaluate in the light of modern genetical and evolutionary knowledge the insect adaptations found there, and to photograph them in their natural habitats. I was also able to compare these with insects found in other tropical forests of which I had had previous experience, such as those of the Belgian Congo, Uganda, and Portuguese East Africa.

Before attempting to analyse my findings it must be stated that after only a few weeks I became acutely conscious of basic differences between Brazilian insects and those I had studied in other parts of the world. These differences may be enumerated as follows: firstly, that in a given habitat of equal area there appeared to be a greater number of species; secondly, that amongst these species, a high proportion fell into the category of extreme specialization; thirdly, that species with more than one line of defence were frequent; and lastly, that whenever an insect depended upon one line of defence alone, such as crypsis, it had to approach perfection. Even so, I was impressed with frequent examples of perfect adaptations whose defences must have been

FIGURE 1 – Euglyphis braganza *Schaus* (*dead set*). *The use of adaptive colouration. Note patterned hindwing.* (×2)

FIGURE 2 – Euglyphis braganza *Schaus* (*alive*). *Cryptic colouration: use of patterned hindwing.* (×2)

FIGURE 3 – Scanthops falcatara *G. Cryptic colouration. Mantid mimicking dead leaf.* (*Natural size*)

FIGURE 4 – Topana media *Walker. Simple cryptic colouration. Orthopteron copying green leaf with brown blemish.* (×3)

FIGURE 5 – Phylloptera *species. More complex cryptic colouration. Orthopteron copying leaf with mould-like markings.* (*Natural size*)

FIGURE 6 – Cossula notodontoides *Schaus. Cryptic colouration. Moth mimicking a fallen flower.* (*Natural size*)

FIGURE 7 – Lirimiris lignitecta *Walker. Cryptic moth mimicking broken twig.* (×2)

FIGURE 8 – Automolis angulosa *Walker. Simple disruptive pattern. Note alternate black and white stripes. (Natural size)*

FIGURE 9 – Thysania *species. Cryptic plus disruptive colouration. Note black stripe parallel to bark markings.* (×½)

FIGURE 10 – Anophylla magnifica *Schaus. Disruptive pattern—bird excreta.* (×2)

FIGURE 11 – Loxolomia serpentina *M. and W. An example of perfect shading. This species copies a piece of dead and loose bark.* (×½)

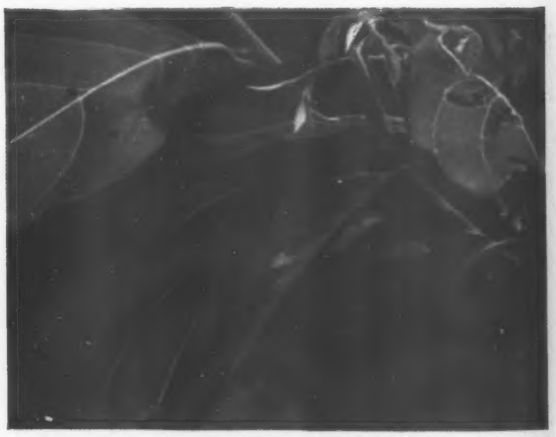


FIGURE 12 – *An unidentified Sphingid larva. A perfect example of counter-shading.* (×½)

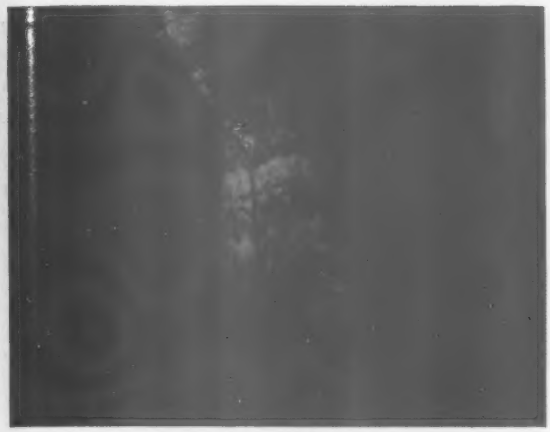


FIGURE 13 – Casandria erratica *D. Jones. Importance of position (abdomen). Note patterned ventral surface.* (×2)

FIGURE 14 – Trosia dimas *Cramer. Warning colouration.* (×2)

FIGURE 15 – Macroneme immanans *Hmpsn. Batesian mimicry. Moth mimicking wasp.* (×2)

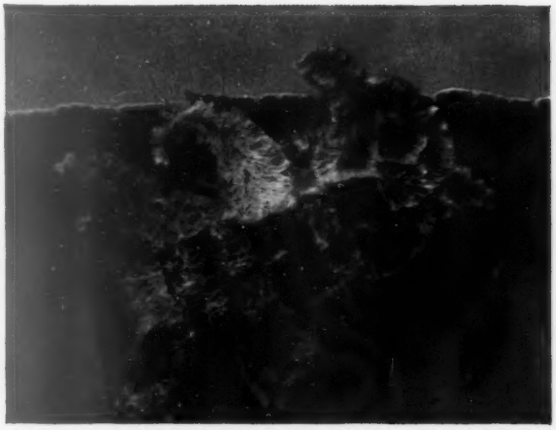


FIGURE 16 – Phoebetron (Euclidae) *larva. Batesian mimicry. Larva mimicking spider.* (×2)

FIGURE 17 – Ommathoptera pictifolia *Walker. Three lines of defence. Display of 'secondary eye-marks'.* (*Natural size*)

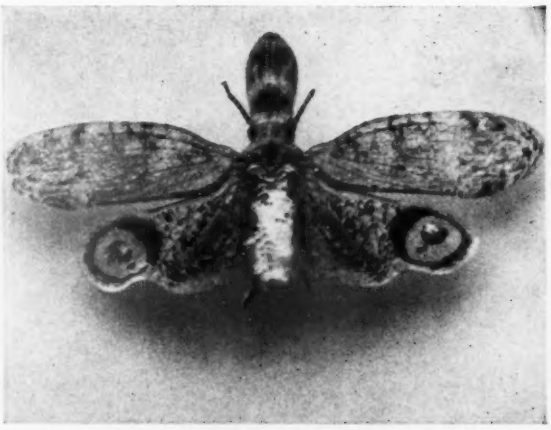


FIGURE 18 – Laternaria phosphorea. *Four lines of defence. 'Horror mask.'* (*Natural size*)

FIGURE 19 – Tanusia brullaei *Blanchard. Orientation to sun.* (*Natural size*)

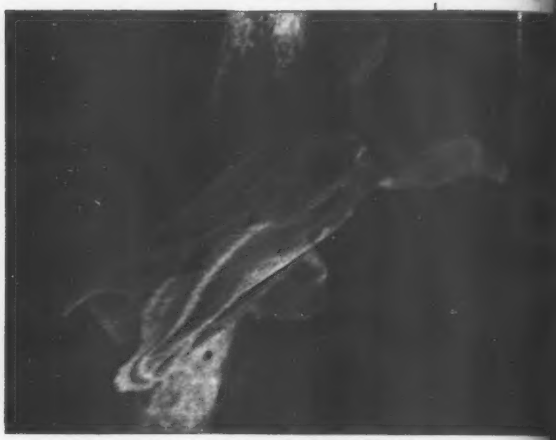


FIGURE 20 – Perola brumalis *Schaus. Importance of pos* (*legs*). (×2)

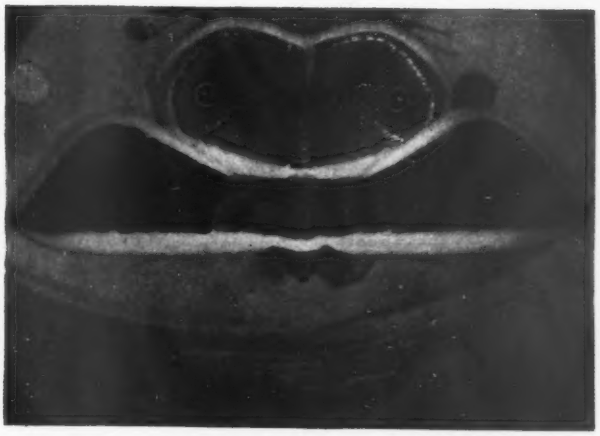


FIGURE 21 – Opthalmophora claudiaria *Schaus.* '*Primary eye-marks.*' *Threat.* (×2)

FIGURE 22 – Megalopygid *larva. The use of hair.* (×

FIGURE 23 – Trichomoplata cassiope *Schaus. The use of bristles.* (×2)

FIGURE 24 – Syssphinx basirei *Schaus. The use of t parency.* (×½)

penetrated sufficiently often to permit one or more additional lines of defence to have been selected. Some species have as many as four, each equally efficient, and each coming into play in succession, according to the seriousness of the situation or the degree of aggressive stimulus provided. Colour and pattern, therefore, go hand in hand with extremely specialized types of behaviour, the one depending on the other, and in insects these must all be inherited and can be to only a small degree, if at all, the result of previous experience, such as affects the behaviour of higher animals.

These impressions—and they must be considered no more than this—would obviously have interesting biological implications if substantiated. One of the objects of this paper is to point out the difficulties of interpreting one's findings quantitatively in order to make a strict comparison between the insect fauna of tropical South America and those found elsewhere. I hope to show that unless one is aware of the whole of the behaviour pattern that a given species follows both by day and by night, it is useless to speculate how 'specialized' it is, or whether a particular pattern is adaptive or otherwise.

A good example of this is provided by *Euglyphis braganza* Schaus. When dead and mounted (figure 1) this very ordinary-looking moth is remarkable for one thing only, namely for having a white pattern on the costa of the hind-wing, an unusual position for patterning. This becomes intelligible only when the insect is seen alive at rest (figure 2). In common with others of the same genus, this moth has a habit of passing the day motionless on tree trunks, with the anterior border of the hind-wings projecting beneath, and in front of, the forewings. The adaptive significance of this hind-wing pattern now becomes apparent: it blends with the white markings of the fore-wings and the advantage of this is that the protruding hind-wings tend to break down the outline of the moth and merge it into the patches of white lichen on which it sits.

Examination of dead specimens or insects caught in traps, tells one very little about the uses to which they put their patterns and nothing about their behaviour, which is an integral part of the whole. I have therefore attempted to depict the insects as I found them in life and to classify their defensive mechanisms according to my interpretation of them. Up to a point, this agrees with the analysis of H. Cott who visited Brazil, amongst other places, and who published his very comprehensive work on adaptive colouration [6] in 1940. H. W. Bates [7, 8], Miles Moss [9], C. L. Collenette [10], W. S. Bristowe [11], P. Poulton, and others have recorded various aspects of Brazilian insect specialization, but most of them have confined their evidence to individual problems. Table 1 classifies the Brazilian insect adaptations as I found them in nature, from the simplest to the most complex. There must, nevertheless, be a direct relationship between the degree of edibility and the extent of the specialization.

TABLE I

Classification of insect adaptations found in Brazil.

1. One Line of Defence
   - (*a*) Simple crypsis (camouflage).
   - (*b*) Disruptive colouration.
   - (*c*) Primary 'eye-marks'.
   - (*d*) Warning or aposematic colouration.
   - (*e*) Müllerian mimicry.
   - (*f*) Batesian mimicry.
2. Two Lines of Defence
   - (*a*) Secondary warning mechanisms (hidden colour signals).
   - (*b*) Secondary 'eye-marks'.
   - (*c*) Flash colouration.
3. Three Lines of Defence
   - (*a*) Crypsis: dazzle and 'eye-marks' (*O. pictifolia*).
   - (*b*) Crypsis: flash: sting (*A. ganascus*).
   - (*c*) Crypsis: distasteful wax: 'eye-marks': 'horror-mask' (*Laternaria phosphorea*).
4. Other specializations (miscellaneous)
   - (*a*) Use of position (i) orientation to sun (ii) legs, body, wings, etc.
   - (*b*) Use of blue scales.
   - (*c*) Use of hair.
   - (*d*) Transparent wings.
   - (*e*) Great size.
   - (*f*) Use of tails.
   - (*g*) Phosphorescence.
   - (*h*) Mirror reflection.

Furthermore, palatability must vary from time to time according to supply and demand. When one species has become extremely abundant because of an unpalatable quality, it is usual to find that some particular predator has adapted its feeding habits to take advantage of the situation. An obvious example of this is seen in the ability of the European cuckoo (*Cuculus canorus* L.) to feed on poison-haired larvae, which are its principal diet: it has a mechanism for regurgitating poisonous hairs as pellets. The actual flesh of many nauseous creatures is, in fact, attractive. A particular defence, therefore, is answered by an equally specialized attack; in Brazil, undisturbed by any recent major climatic change, this has been carried to extremes. Though there is ample

evidence that the South American continent has undergone vertical shifting, there has certainly been no glaciation in Brazil since the Pliocene.

ONE-LINE DEFENCE

With these thoughts in mind, we will consider some of the 'one-line defence' insects that depend on crypsis alone. L. de Ruiter [12] has shown that when European jays (*Garrulus glandarius* L.) were fed on caterpillars that mimic twigs, together with a number of actual twigs from the trees on which they were found, the birds' interest was proportionate to the number of insects presented compared with twigs. It therefore follows that—as in any forest there are more leaves, dead and living, than twigs, and more twigs than boughs or trunks—leaves would offer more unsuccessful encounters than twigs, even having regard to the surface areas presented. It is not surprising, therefore, that a very large number of species of insects copy both dead brown leaves and living green ones. An excellent example of the former is *Scanthops falcatara* G., the Brown Mantid (figure 3). It escapes predation by its similarity to a dead leaf, and also gains advantage from the deception by permitting other insects on which it feeds to approach it unawares.

Many species of moth copy dead leaves by presenting a longitudinal stripe from tip to tip, which simulates the midrib. Others have wings with edges that are 'eaten out' but still successfully obey the laws of aerodynamics. In the pattern many include copies of leaf blemishes, holes, and mildews which appear as brown, transparent, or whitish spots. The moth *Meticulodes spongiata* Guen., inverts the costa of its forewings and rolls them up into hollow tubes, giving the impression of a shrivelled leaf. I also found a single specimen of a small Geometrid moth which held its brown wings asymetrically when at rest; the tip of one turned up and the other down. On touching this moth, to my surprise it dropped off, and, revolving rapidly, it went spinning away like a sycamore seed.

Simple green-leaf mimicry is well demonstrated by the Orthopteron *Topana media* Walker (figure 4) and in addition brown blemishes are to be seen upon it. In a *Phylloptera* species (figure 5) white mould marks are evident and it is extremely difficult to find except at night. A large number of Lepidoptera in Brazil look like green leaves, but the majority have additional markings for disrupting the outline or presenting blemish-like spots on them. The moth *Cossula notodontoides* Schaus (figure 6) mimics a fallen flower and sits exposed: it is a rare species. Dead wood and twigs are frequently copied. One of the most perfect examples of this is the moth *Lirimiris lignitecta* Walk (figure 7). The broken end of a twig is sculptured to perfection by the modification of the head and thoracic structures.

Simple disruptive patterns, which break up insect outlines, are common in the tropics. The bright vertical sun permits the exploitation of alternate black and white stripes as is seen, in its simplest form, in *Automolis angulosa* Walk (figure 8). An extension of the same principle is found in many moths which combine disruptive stripes with crypsis. All these spend the day at rest in such a way that the stripes run parallel with the vertical lines on the bark (figure 9). In certain species this demands that the moths sit sideways on the trunk, which they unfailingly do. This then, is another example in which a specialized pattern requires a specialized behaviour. Figure 10 shows a Geometrid moth with a beautiful and complicated disruptive pattern. It sits exposed on the surface of green leaves, looking like old bird-excreta; many species mimic this, some to an incredible pitch of perfection. Specialized shading is seen in the moth *Loxolomia serpentina* M. and W. (figure 11) which spends the day at rest, looking like a piece of loose bark. Countershading is beautifully demonstrated by an unidentified Sphingid larva (figure 12). The vertical tropical sun also permits it to have bright white splashes which simulate the light reflected from the shining leaves of its food plant. This larva always sits upside down; when the dorsal surface is deliberately placed uppermost it becomes conspicuous.

All the insects so far discussed and depicted have depended for their survival on crypsis—perfect camouflage. Another one-line defence mechanism is found in insects that reproduce 'eye-marks' as a conspicuous feature of their pattern. Those that we are about to discuss here have them permanently on view, often on the forewings. I therefore refer to these as primary eye-marks, and this defence and its uses must be kept distinct from another in which insects have life-like 'eyes' but normally keep them concealed, exhibiting them only on attack. These will be referred to later under the heading of secondary eye-marks.

Primary eye-marks, which are frequently of small size but nevertheless perfect, have two distinct uses. Firstly, the larger ones may serve to threaten and frighten away predators; secondly, the smaller ones act as 'deflection spots.' For the

latter use they must always be situated distally, and as far away as possible from the vital areas of the body: the 'eyes' then invite attack on unimportant structures. This mechanism is found commonly among Brazilian butterflies, usually on the underside of the wings and hence only of use whilst at rest. Figure 21 shows the moth *Opthalmophora claudiaria* Schaus in which the eye has been selected to a very high degree of perfection, complete with pupil light reflection. In this instance it is probable that it serves as a threat mechanism.

Another threat mechanism, usually referred to as warning or aposematic colouration, is common among Brazilian insects. It leads to some of the most complicated and sophisticated insect relationships in the world. Its general principle is precisely the opposite to that of crypsis: it depends on revealment instead of on concealment. In its simplest form an insect with some unpleasant quality, such as nauseous taste or smell, makes itself conspicious with the object of reminding its predators, by association, of previous unpleasant encounters. The moth *Trosia dimas* Cramer (figure 14), with its red domino-like pattern, is an example of this. I found it had a most unpleasant taste and smell.

It is not therefore surprising that many insects that have distastefulness in common have been selected to have similar patterns. They all wear a conspicuous and similar uniform. An unpleasant experience gained from one species will give protection to others. Usually the warning colours are black and red, or black and yellow, sometimes white. This defensive mechanism, in which numbers of different distasteful species, similar in appearance, fly together, is known as Müllerian mimicry.

An extension of the same principle is found in which certain species of insects, whilst themselves palatable, masquerade under the warning guise of others that are not and fly with them. These are referred to as Batesian mimics. It is the usual rule, but depending on the degree of distastefulness of the model, that in any assemblage, the Batesian mimics must not outnumber the Müllerian, or the system would break down. This situation is found commonly on the Amazon, where large numbers of different species of butterfly congregate together on the damp tracks used by humans.

Another type of Batesian mimicry is found in which warning colouration plays no part. Many different kinds of insects mimic, to a quite remarkable degree, specific creatures universally accepted as dangerous. The moth *Macroneme immanans* Hmpsn., for example, mimics a black ichneumon wasp with which it flies (figure 15). Others, both moths and larvae, copy spiders. When I first found the caterpillar of a *Phoebetron* (*Euclidae*) species (figure 16) I could not decide immediately even to which order it belonged. It has eight fleshy appendages which if torn off regenerate on moulting, and it sits exposed like a large spider on the surface of a leaf. The male Psychid moth *Oiketicus kirbyi* G. has an enlarged first pair of legs and a greatly extended abdomen which, on attack, it telescopes in and then out again: the overall effect is scorpion-like. Several species of beetle mimic ants with which they live, thereby gaining protection. In all these Batesian mimics it is not enough that colour and pattern should match those of the model. The mimic must conform also to the specialized behaviour patterns.

One last and previously unrecorded example of Batesian mimicry must be given. *Cecropia* tree species are common in Brazil and are characterized by having hollow trunks and an association with ants, which frequently cover the leaves. Whilst working in the Corcovado Forests with E. P. Wiltshire we found a mass of oily and heavily spined caterpillars on the underside of a leaf. They were the larvae of *Gynaecia dirce*, a well-known Brazilian butterfly. They appeared to be immune from the attack of ants, which abounded everywhere. These larvae have the usual number of legs and walk in the usual manner. On disturbing these larvae by picking the leaf on which they were resting, we noticed another larva of similar appearance and pattern among them, but which 'looped' on walking. It was the larva of an entirely different species, a Geometrid moth. On closer examination we found that the spines of the butterfly larvae were copied by fleshy outgrowths on the Geometrid larvae. Unfortunately, the Geometrid larva died and no other examples were found, as it was too late in the season. Presumably it gained protection whilst feeding on the *Cecropia* by living with the larvae of this protected butterfly. It is interesting to reflect on how the larvae of a Geometrid moth can succeed in establishing itself in the midst of those of a *Nymphaline* butterfly.

Every survival mechanism described so far has relied on one line of defence only. When this is penetrated, the insect's defensive repertoire is exhausted and death is the inevitable result.

TWO-LINE DEFENCE

Many insects have developed two or more lines of defence. In its simplest form this is seen in

various threat or warning reactions which come into play when the crypsis has failed and the insect is attacked. Commonly the hind-wings or abdomen are exposed and the colours red or yellow are flashed at the predator; this always goes with a special behaviour pattern, such as rhythmic displays or feigning death. Some species, such as *Syssphinx molina* Gram. display a single black spot in the middle of a brightly coloured area. This is the most likely origin of the hind-wing eye-mark in which an eye is copied to a quite remarkable degree of perfection in certain South American moth genera, such as the *Automeris*. I refer to these as secondary eye-marks. D. Blest [13] has shown in aviary experiments that the acceptance or refusal of these moths as an article of diet by birds is decided by the perfection of the eye-mark. In my opinion it is likely that the perfect eye has been built by natural selection in a series of stages, probably commencing with a plain black spot. We have evidence from the British Lepidoptera that a single gene can so alter a circular black spot that it appears as a shaded area with a thin rim of black retained around the circumference (in a form of *Arctia caja*). A second gene is responsible for a contrasting ring running round the outside of this, and yet another for the light reflection seen on the 'pupil' (in *Saturnia pavonia* L.). No doubt several genes become linked by natural selection, the whole forming a super-gene which in effect works as one unit. All gradations of eye-marks are found in Brazil, the majority having great perfection of detail.

I was able to carry out a short series of releases of *Automeris* moths and other species with secondary eye-marks. These were undertaken at daybreak, which is the main feeding time for the majority of birds. I was greatly surprised to see that, when discovered, the moths were seized and carried away in a flash: they appeared to have no opportunity of bringing their eye-mark mechanism into play. Subsequently I was able to follow a bird and noted what happened. In order to peck and eat the moth it was, of course, necessary to drop the insect on the ground; the moth then flashed its eyes and the bird recoiled and the moth escaped. On a further occasion I saw an *Automeris* moth escape after being carried away, but I was unable to see the details of how it managed it. I have little doubt that this is the normal method of use of the secondary-eye mechanism. Strangely I found no *Automeris* species showing beak marks.

Another two-line defence mechanism, flash colouration, is found commonly, particularly among Brazilian Acrididas, Cicadidae, and Lepidoptera. The forewings are cryptic, but when the insect takes flight the brilliant scarlet, yellow, or blue hind-wings command attention. On alighting, they become inconspicuous again and merge with their background.

THREE OR MORE LINES OF DEFENCE

Many insects have developed more than two lines of defence. The highly cryptic Orthopteron, *Ommathoptera pictifolia* Walker, at rest, looks exactly like a dead leaf. On being disturbed it quivers its wings rapidly, exposing the multi-coloured underside, and this produces a confusional effect. Finally it throws its wings open and exposes two eye-marks (figure 17).

The Sphingid moth, *Amplypterus ganascus* is cryptic, with a disruptive pattern. When attacked it exposes its red hind-wings, and if picked up this moth 'stings': I was greatly surprised to receive a most painful prick the first time I handled one. On examination I found that it had greatly enlarged tibial barbs, which no doubt discourage insectivores such as monkeys.

Probably the insect with the largest repertoire of defensive mechanisms is the Sulphorid bug *Laternaria phosphorea* (figure 18). It is cryptic when at rest but has a chitinous extension to its head. This can only be considered a 'horror mask' and is very like a diminutive alligator head, complete with dummy teeth and eye prominences. When seized, it exposes two large eye-marks on its hind-wings. Lastly, its abdomen is covered with a mould-like wax which probably makes it distasteful. We can assume that each of these defensive mechanisms has been developed during different periods in its past history when new predators demanded new defences. It is, in fact, unlikely that any species can build up more than one specialization at the same time [14]. Though so extreme in having so many lines of defence, it is probable that *Laternaria* has survived because of the absence of gross environmental changes which frequently must eliminate too highly adapted individuals, as happened to the Mastodon.

The classification we have adhered to up to now has the advantage of permitting us to envisage clear-cut reactions that are brought into play in a particular sequence. There remain, however, a miscellaneous series of highly specialized adaptations, some of which are alone sufficient to give protection: others take their place as only part of a behaviour complex.

OTHER SPECIALIZATIONS

Great heat under a vertical sun produces its own complications. If one releases small Geometrid moths in the middle of the day over a bare and rocky area devoid of shade, but having trees at fifty yards distance, many collapse and fall dead to the ground: they are probably dehydrated in flight. It is not surprising that methods to avoid this danger have arisen. Many species at rest orientate themselves to the sun in such a way that they present their minimum surface area towards it, and hence their wings are always in the shade. For this reason it was most difficult to photograph *Tanusia brullaei* Blanchard, the Green Leaf Insect (figure 19), without the aid of a mirror, because it always kept its long axis vertical to the sun. Several *Thyrididae* and *Draconiae* species of moth use the same mechanism and pass the day standing 'on tiptoe' thereby throwing the minimum shadow and absorbing the least heat. Another mechanism, not previously described, is probably connected with deflection of heat or sunlight. Many insects that have to pass the day in direct sunlight have a blue or violet colouration: this is seen in the highest form in the Morpho butterflies. It is common, however, to a lesser extent in many moths that fly by day or are exposed at rest to sunlight. An interesting point is that the blue colouring, if limited in extent, is always present proximally over the vital areas and not over the distal portion. The blue-violet colour probably deflects certain rays of the sun and hence protects the insect [15].

Many Lepidoptera, such as *Perola brumalis* Schaus, make use of a greatly enlarged first pair of legs and spend the day with them sticking out conspicuously in front, giving a most unmothlike appearance (figure 20). Normally these legs are either entirely hidden beneath the insect or blend with the background on which it is sitting. Others, in widely differing genera, throw the abdomen over their head, and in this position they pass the day. In the majority of *Heterocera* (Moths) the abdomen lies concealed beneath the wings, with the ventral surface held tight against the resting surface. Examples of the former are found in the species *Megastes grandalis* Guen. [15] and *Casandria erratica* D. Jones (figure 13). In this last species it is of the greatest interest to note that, contrary to all other Lepidoptera known to me, it has a highly developed cryptic pattern on its ventral surface, which is exposed. This must have been built up by natural selection in association with the extraordinary position of abdominal dorsiflection in which it rests. In some species this strange position probably serves to keep the head and thorax in the shade of the abdomen (*Cicinnus* species). In others (*erratica*) it may be that if pecked by a bird, the body, not being found in the usual position beneath the wings, escapes injury.

Hairs and bristles are made use of throughout the Brazilian Insecta. Many lepidopterous larvae are covered with barbs or hairs of great length, as in the *Megalopygidae* (figure 22), and in many species these are highly poisonous. The moth *Dirphia hortensis* when attacked feigns dead and exposes its red and loosely haired abdomen. In the same way, the wax covering of certain specialized Hemiptera has been greatly accentuated, and this undoubtedly acts as a deterrent to predators. In the cryptic Cicada *Phoenix varigata* waxy strands cover the abdomen. Strangely, this wax is itself the food of a micro-lepidopteron, whose larvae feed on it. In other species (figure 23) hair bristles are developed around the periphery and these probably keep off the ants which swarm everywhere in Brazilian forests. In some instances, hair may serve to merge an outline into its background.

Transparent wings are made use of defensively in many ways. In the depth of the forest many species of butterfly of widely different genera show convergence of pattern, having transparent wings with black markings. They are all most difficult to follow in flight in the deep shade. Examples of this are *Zeonia licursis* Fab., and *Ithomia dryma* Hbn. *Syssphinx basirei* Schaus (figure 24) has transparent windows in the wings, which when at rest give a disruptive pattern when viewed from beneath.

Another special modification is the use of 'tails.' *Copiopteryx sonthannaxi* André [15], of which I found only one specimen, has two tails of great length, terminating in small light-coloured pennants, which in flight appeared to make a rustling noise. It is active at dawn and the tails are probably of service in deflecting attacks by bats, which are extremely numerous at that time.

So-called phosphorescence is frequently found in the Coleoptera: 'fire-flies' abound in all the forests. A *Pyrophorus* species which was noted by Darwin [1] is worth special mention. This Click-beetle has two luminous spots that can be seen at night at a distance of several yards. Its two 'headlamps' possibly serve as a threat, or alternatively are bright enough to act as a visual aid in darkness. They are unlikely to be a sex attractant, as in some other beetles.

Brazil can probably boast some of the largest

Lepidoptera and heaviest Coleoptera in the world. The noctuid moth *Thysania Agrippina*, has a wing span of just under 27 cm. Great size must confer advantages at times by shifting the species out of the range of certain predators.

I came across one other protective device not previously described among insects, but well-known in fish [16], namely the use of 'mirror reflection.' The pupa of *Mechanitis lysimnia* Fab. presents such highly mirrored surfaces that it reflects its surroundings wherever it pupates. By night it is easy to find with a torch because it appears as a bright spot of light.

CONCLUSIONS

I have given an interpretation of some of the more extreme defence mechanisms found in insects which I came across during seven weeks spent in Eastern Brazilian forests. During this period over 5000 selected specimens were caught and are now in the British Museum. I have suggested that extreme adaptive specialization may be commoner in South America than in the comparable African tropics. Furthermore, it is possible that there are more insect species in a given area of this region than elsewhere. Why could this be so in Brazil? Firstly, the most likely explanation is that the insects are based on a very large flora. There are four to five hundred tree species in eastern Brazil alone: each presents different buds, leaves, and trunks, and each offers a potential niche. The trunks alone can be white (as noted by Darwin [3]), red, or blue-green, according to the lichen species which cover them: each colour is exploited. Secondly, ruthless natural selection is ensured by a large number of insect eaters. There are three to four hundred species of birds in eastern Brazil alone, apart from lizards, frogs, monkeys, and other predators that rely on sight. Thirdly, the country has undergone no recent gross environmental changes such as glaciation: extreme adaptations have therefore not been eliminated. Finally, a variable countryside with suitable mountain ranges has been able to offer alternative accommodation during minor climatic changes.

Though equally complex and similar mechanisms undoubtedly exist in the African tropics and elsewhere, to me they appear not to occur so frequently. If this is so, an increased biological pressure occurring in a country offering an almost infinite number of biological niches may account for the quite incredible insect fauna of Brazil.

ACKNOWLEDGMENTS

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REFERENCES

[1] DARWIN, C. 'Journal of Researches'. 1845.
[2] DARWIN, C. 'The Origin of Species'. (Sixth edition). 1859.
[3] DARWIN, C. 'The Beagle Diary'. 1839.
[4] WALLACE, A. R. 'Contributions to the Theory of Natural Selection'. London. 1870.
[5] DARWIN, C. and WALLACE, A. R. *Proc. Linn. Soc., Lond.*, III, 1858.
[6] COTT, H. 'Adaptive Colouration in Animals'. 1940.
[7] BATES, H. W. *Trans. Linn. Soc., Lond. (Zool.)*, 23, 1862.
[8] BATES. H. W. 'Naturalist on the River Amazon'. 1892.
[9] MOSS, M. *Novit. zool.*, 27, 1920.
[10] COLLENETTE, C. L. *Proc. zool. Soc., Lond.*, 1935.
[11] BRISTOWE, W. S. *Trans. R. ent. Soc., Lond.*, 475, 1924.
[12] DE RUITER, L. *Behaviour*, 4, 3, 1952.
[13] BLEST, D. *Ibid.*, II, 209, 1957.
[14] HALDANE, J. B. S. *J. Genet.*, 1958.
[15] KETTLEWELL, H. B. D. *Life Magazine*, November 1958.
[16] HARDY, A. C. 'The Open Sea', Part 1, p. 226. Collins, London. 1956.

Science in Israel

The creation of the independent State of Israel in 1948 was the culmination of long years of effort by leading Zionists throughout the world. Their leader was Chaim Weizmann, a unique combination of statesman and scientist. To his skill and pertinacity in the first role Israel owes its existence; from his vision in the second stems a great deal of the country's remarkable practical achievement during the past ten years. In the main, the world's statesmen have shown reluctance to be persuaded that science and technology are not merely interesting and important but essential to modern civilization: Israel may count herself fortunate in that her first leader not only had no need to be persuaded to accept this fundamental truth but lost no opportunity of convincing others of it. It is particularly fitting, therefore, that his memorial should be a scientific institute, at Rehovoth, that has been described as one of the most effective research complexes in the world.

The Weizmann Institute in its present form dates from 1949: the funds for its creation were subscribed to mark Weizmann's 70th birthday in 1944. Rehovoth was already an established scientific centre, however, for in 1934 a chemical research laboratory, the Daniel Sieff Research Institute, had been built; in this Weizmann himself found relaxation in research in his later years. This venture was a great act of faith, for at that time the whole future of Palestine was uncertain. Nevertheless, Weizmann saw clearly that if the independent State of which he dreamed was to survive, the help of science and technology would be indispensable. Events have proved how right was his bold conception of establishing a centre, primarily for fundamental research, in what was then an inhospitable wilderness.

Today the Weizmann Institute, beautifully set among trees and gardens, has on its staff 180 scientists and nearly 400 assistants, the great majority of them born in Israel. Its nine departments, well housed and well equipped, cover such varied fields as applied mathematics, nuclear physics, electronics, X-ray crystallography, organic chemistry, experimental biology, and biophysics. Where so much is good it is difficult to select for special mention specific examples of work in hand, but certainly the cancer research, the production of heavy oxygen, the preparation of large-ring conjugated systems, the investigation of the nidation of ova, and the work on polymers are all excellent. The outlook at Rehovoth is international, and its senior scientists travel widely to visit centres of research abroad. Conversely, increasing numbers of scientists from abroad are visiting Rehovoth to learn at first hand about research in progress and, not infrequently, to take some part in it: for such visitors excellent facilities exist. The Institute is not a teaching establishment, but in 1958 a post-graduate school was opened.

Impressive though the achievements of the Weizmann Institute are, it represents only part of a determined national effort to promote science. There are two national institutes for higher education, the Hebrew University at Jerusalem and the Technion at Haifa. The first of these has a remarkable history. It was first established on Mount Scopus in 1925, the fulfilment of ideas that were first put forward as early as 1882. Since the War of Independence in 1948 Mount Scopus has been in Arab territory, and although a fortnightly convoy is allowed to go through to the university, no more than a caretaker staff can be maintained. After some difficult years of existence in buildings scattered about Jerusalem, it was decided to build a new university on Girath Ram, a hill on the west of the city. Here many fine new buildings have been erected during the last four years, and more are planned. Of the total of rather more than 4000 students, about 1750 are in the faculties of science, agriculture, or medicine. The building of this completely new university is a remarkable achievement.

The Technion at Haifa is beautifully situated on Mount Carmel, and is the oldest scientific institution in Israel. Begun in 1912 as an institute for applied science, it was not completed in its original form until 1923. Now it has 3000 students —studying for degrees in all the principal branches of engineering—and a dozen new buildings have been built in recent years. The Technion serves the dual purpose of supplying trained engineers for Israel's industry and of carrying out industrial research: in recent years part of the latter has been done under contract for other countries—another example of Israel's international outlook.

The antibiotics in microbiology

E. P. ABRAHAM

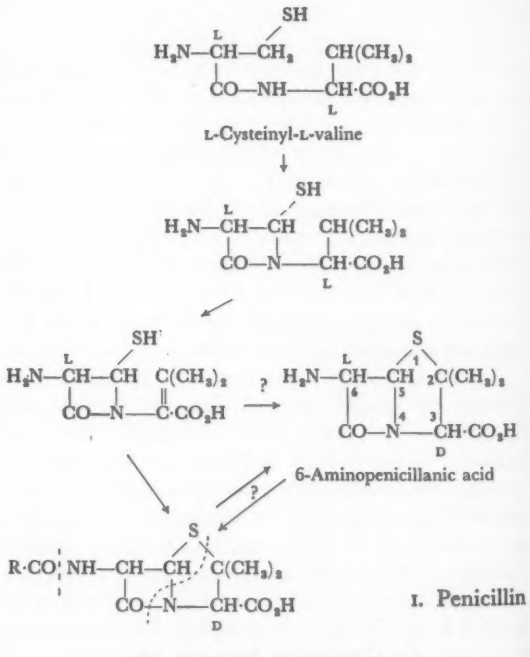
The spectacular therapeutic success of penicillin, and subsequently of other antibiotics, naturally led to the main concentration of effort in this field being directed towards practical application. Now, however, threads of continuity can be discerned where at first only a series of separate achievements was apparent, and results of fundamental microbiological interest have been achieved. The biosynthesis of antibiotics can be linked with that of other cellular constituents, and some light has been thrown on their mode of action.

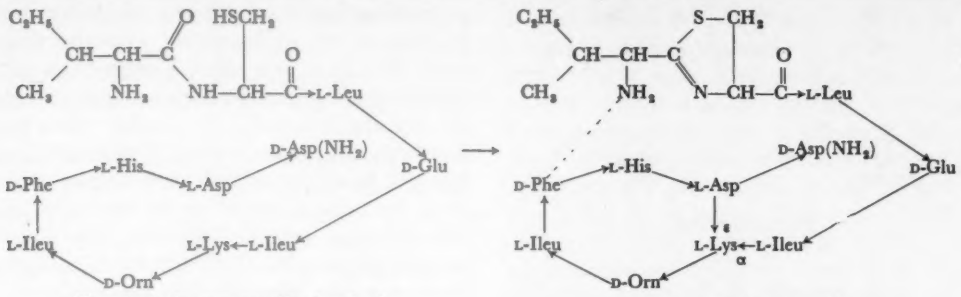
Nearly twenty years have now passed since the discovery of the chemotherapeutic properties of penicillin by Florey, Chain, Heatley, and their colleagues and fifteen since the first publication on streptomycin by Schatz, Bugie, and Waksman. In that time we have passed from sporadic and accidental encounters with antibiosis by individual microbiologists to the systematic screening of many thousands of micro-organisms by the pharmaceutical industry, with the primary object of obtaining new drugs of clinical value. These expensive large-scale searches for therapeutically useful antibiotics have led to the discovery of chloramphenicol, the tetracyclines, erythromycin, and other substances that act as powerful antibacterial agents *in vivo*, and they have produced compounds of value against certain fungal infections. In some laboratories they are also aimed at the discovery of substances that will selectively inhibit the growth of tumour cells or viruses, though there is so far little evidence to indicate that they will have much success in this field.

The discovery of new antibiotics with useful chemotherapeutic properties has become a formidable task. In one survey of 10 000 actinomycetes from the soil some 2500 were found to produce antibiotics, but only ten of these substances were new and only one was clinically effective [1]. We are perhaps approaching a stage when the appearance of a new type of chemical structure in this field will be comparatively rare. It may thus be appropriate to consider the broad significance of the intensive work of the last two decades.

The striking achievements of the antibiotics in clinical medicine are common knowledge. Many bacterial infections that were once troublesome, dangerous, or even fatal can now be overcome. Were it not for evidence that the suppression of some types of infection facilitates the growth of others, and for the emergence among certain species of bacteria, in particular the staphylococci, of strains that are resistant to antibiotics, there would be good reason for satisfaction in this sphere of chemotherapy. However, the introduction of these particular substances into medicine involved no major additions to scientific theory. Once the fact that an antibiotic might show chemotherapeutic properties had been established, the field was exploited by skilled and systematic work of a largely empirical kind. Gratifying though the practical results have been, they yet seem intellectually unsatisfying.

Less pragmatic studies of the antibiotics are





Hypothetical precursor of bacitracin A containing only α-peptide bonds

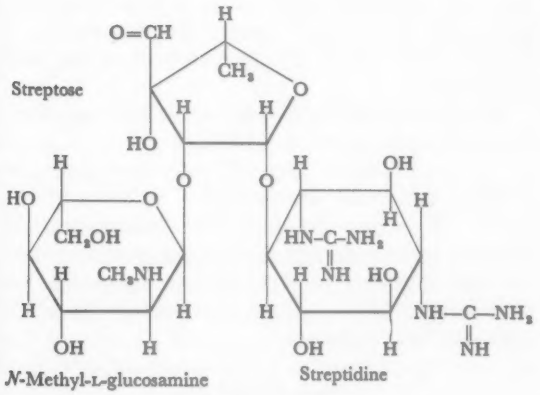
II. Bacitracin A

now beginning to produce results. A great number of microbial products have been revealed which would doubtless be still unknown had they not been labelled by their property of antimicrobial activity. The structures of many of these products have been determined, and a basis has thus been established for an attack on two problems that are of general interest to microbiology: how are these substances formed in the cell, and how do they exert their characteristic activity?

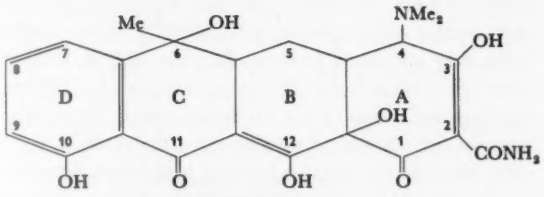
BIOSYNTHESIS OF ANTIBIOTICS

The antibiotics comprise a heterogeneous collection of compounds. A casual inspection, however, is sufficient to reveal that many of them can be grouped into families whose members are structurally related. *Penicillium chrysogenum* may synthesize a series of penicillins (I) which differ only in the side-chain (R), the main compound formed depending largely on the precursor of the side-chain that is added to the culture medium. *Bacillus licheniformis* forms bacitracin A (probably II) and a number of similar peptides in which valine is substituted for isoleucine. *Streptomyces griseus* produces not only streptomycin (III), but mannosidostreptomycin, in which a residue of mannose is linked to the L-glucosamine fragment of the molecule, and hydroxystreptomycin, in which the methyl group of the streptose fragment is replaced by a hydroxymethyl group. Other species of *Streptomyces* produce the various tetracyclines (IV); these include aureomycin and terramycin, which differ in their substituents at positions 5 and 7, and demethyltetracyclines, which have no methyl group at position 6. *Streptomyces* species also produce a group of antibiotics that are related chemically to erythromycin (V). These substances all contain large lactone rings, known as macrolides, attached to unfamiliar sugars or amino sugars (R_1 and R_2 in V).

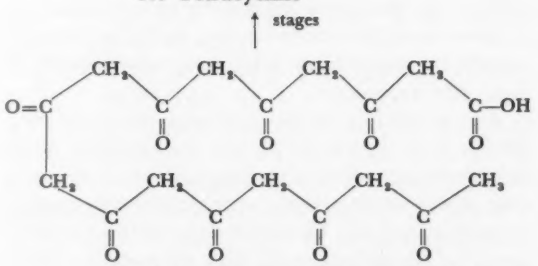
The different members of a single antibiotic family are often produced by the same species of organism, but this is not always so. A species of the genus *Cephalosporium* produces a hydrophilic



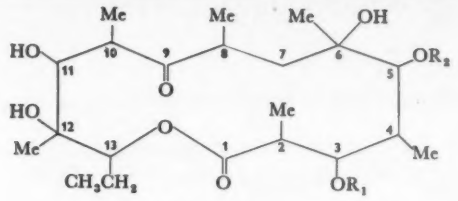
III. Streptomycin



IV. Tetracycline



Hypothetical precursor (octaketostearic acid)



v. Erythromycin

penicillin, cephalosporin N, in which the side-chain is derived from D-α-aminoadipic acid, and a related substance, cephalosporin C, which does not contain a normal penicillin nucleus [2]. When we consider structural fragments of the antibiotics and not the complete molecules, relationships are more widespread. For example, the bacitracins contain a thiazoline ring which is structurally similar to thiazole rings in a very different antibiotic, micrococcin, obtained from a micrococcus and from *Bacillus pumilus* [3].

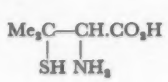
These examples illustrate the tendency for micro-organisms to produce numerous variations on certain structural patterns. It now appears that the patterns themselves, despite their striking differences, are commonly built from only a few types of structural unit: amino acids, acetate, and simple sugars. In this respect the biogenesis of antibiotics resembles that of a great number of other natural products.

Many antibiotics derived from bacteria and actinomycetes are polypeptides, and are thus clearly derived from amino acids, but the biosynthesis of these substances presents problems which have not arisen in connection with animal peptides and proteins. The antibiotics may contain amino acid residues which differ structurally from any of those present in proteins, and also residues which have the D-configuration. Their compositions may vary, in some cases, according to the amino acids present in the culture fluid, and their peptide chains are formed into large rings or are terminated by unusual groups. Free D-amino acids in micro-organisms appear to originate from enzyme-catalysed racemization, but it is uncertain whether these amino acids are incorporated as such into peptides.

An interesting member of this group of antibiotics is bacitracin A [2, 4]. Its molecule could arise from two types of cyclization in a dodecapeptide whose amino acids were joined by classical α-peptide bonds. Condensation of the carboxyl group of an aspartic acid with the ε-amino group of lysine would give the ring of six residues, while a condensation in an *N*-terminal isoleucylcysteine portion of the chain would give the thiazoline ring. But it is not clear whether the cells use specific mechanisms for making other members of the bacitracin family, or whether these peptides are formed because valine is imperfectly distinguished from the structurally similar isoleucine at a site where synthesis occurs. Our present knowledge of peptide hormones from the higher animals suggests that here genetic factors uniquely determine the structure of the molecule. For example, different insulins exist in which valine replaces isoleucine, but each appears to be synthesized only by a given species of animal.

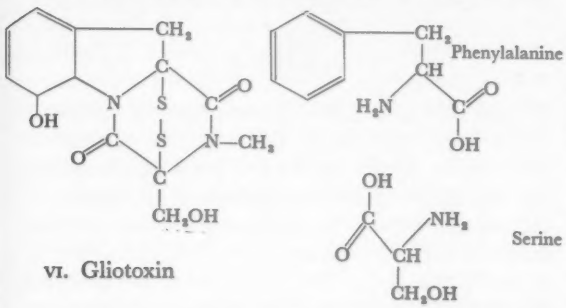
With other antibiotics the possibility of dividing the structure into amino acid residues is less obvious but nevertheless clear. The dissection of penicillin into L-cysteine, D-valine, and an acid $R.CO_2H$, as shown in I, provides a well-known example. Studies by Arnstein and others have left no doubt that this dissection reveals the types of unit from which the antibiotic is formed in *P. chrysogenum* [5]. L-Cysteine labelled with ^{35}S, ^{15}N, and ^{14}C has been shown to be incorporated intact into the molecule. Identification of the origin of the D-valine residue has proved to be a more difficult problem, but it now seems likely that an early step in the synthesis is the formation of L-cysteinyl-L-valine: the latter, or an acyl derivative of it, is thought to condense to yield a β-lactam. Dehydration of the L-valine residue could then yield an intermediate from which the penicillin nucleus (6-aminopenicillanic acid) or a penicillin might be reached by addition of sulphur to the double bond, the configuration at the reformed centre of asymmetry (C_3) being dextro. 6-Aminopenicillanic acid has been obtained from the culture fluid of *P. chrysogenum*, but its isolation does not establish whether it is the immediate precursor of a complete penicillin structure or is formed from the latter by removal of an acyl group [6].

Enzymic systems which can effect such reactions have yet to be isolated, although an interchange of acyl side-chains between one penicillin and another has been demonstrated in cell-free extracts. It is notable, however, that the mould does not make a special amino acid, penicillamine, for the synthesis of this strange molecule but manipulates compounds that are normally available. D-Penicillamine



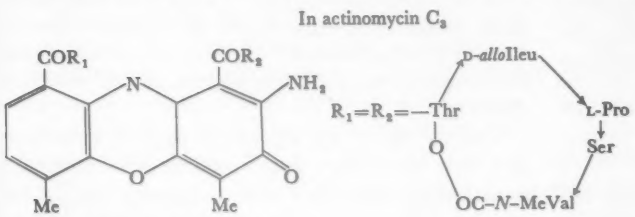
is a characteristic product of the chemical degradation of penicillin, and provides a starting point in a rational laboratory synthesis, but it does not appear to be formed in nature as an independent unit.

Further examples of antibiotics that arise from the condensation of amino acids are easily found. Gliotoxin, which was first isolated from a fungus more than twenty years ago, has recently been shown to have the structure VI [7]. In this we see

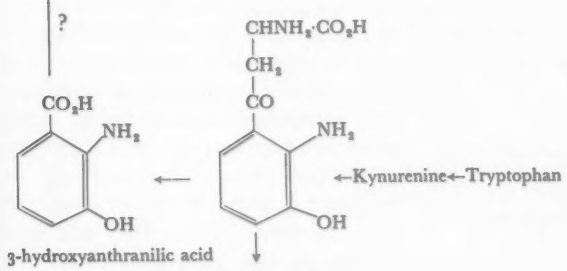


VI. Gliotoxin

the skeletons of phenylalanine and serine, to which two sulphur atoms have been attached in a unique manner. When added to the culture fluid, labelled phenylalanine is incorporated intact into the molecule [8]. The actinomycins (VII)—a family



VII. Actinomycin



Ommochrome pigments

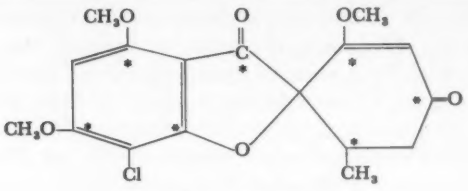
of antibiotics with anti-tumour activity, produced by *Streptomyces antibioticus*—consist of a phenoxazone chromophore linked to two variable though related peptides (R_1, R_2) containing large lactone rings [9]. The phenoxazone can be dissected into two molecules of 3-hydroxy-4-methylanthranilic acid. Hydroxyanthranilic acid itself can be formed, by a series of enzyme-catalysed reactions, from the amino acid tryptophan. A derivative of hydroxyanthranilic acid is known to be the precursor of phenoxazone structures that are present in a group of pigments (the ommochromes) occurring in the eye of insects [10]. It is possible that a similar pathway, together with a process for the addition of a methyl group, leads from tryptophan to the actinomycin chromophore [9.]

Inspection of the structures of a variety of other antibiotics has led to the suggestion that they are derived from acetate. Several types of structural feature are indicative of this biogenetic origin. One is the distribution of oxygen or double bonds in such a way that the compound could be formed by acceptable modifications of poly-β-keto acid:

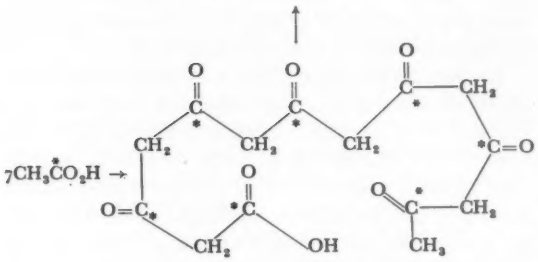
$$2x CH_3.CO_2H \rightarrow x(—CH_2.CO.CH_2.CO—)$$

This type of process was suggested by Collie, as long ago as 1906, as being responsible for the formation of certain phenolic compounds. Collie's hypothesis was developed by Robinson and others [11], and in recent years its importance has been experimentally established by Birch in the case of phenolic plant products and mould metabolites [12]. A straightforward and elegant example of its application is provided by griseofulvin, an antibiotic obtained from several species of *Penicillium*, which has been used systemically for the treatment of fungal infections in man. When griseofulvin is produced in the presence of acetate whose carboxyl group is labelled by ^{14}C, the distribution of radioactive carbon in the molecule is precisely that required by the scheme shown (VIII) [11]. A more complicated example is provided [12] by the tetracyclines (IV). Here the experimental evidence is incomplete, but it supports the view that rings D and C, and part at least of ring B, are formed from acetate.

The skeleton provided by a poly-β-keto acid often requires the addition of extra groups or atoms, such as methyl or chlorine, before the final structure is reached. The frequent presence of extra methyl groups raises no obvious difficulty, for the *S*-methyl group of methionine is known to be transferable, in the presence of appropriate enzyme systems, to oxygen, nitrogen, or carbon in other compounds.

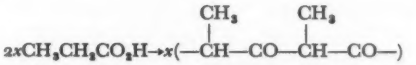


VIII. Griseofulvin



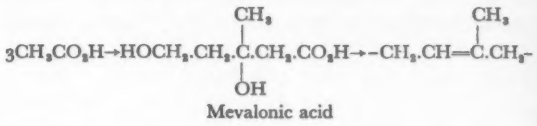
The methoxyl groups of griseofulvin, and probably also the methyl at C_6 of tetracycline, originate in this way.

The structures of the lactone rings of the erythromycin group of antibiotics are consistent with the assumption that they are formed from poly-β-keto acids. In erythromycin itself, however, methyl groups appear on the even-numbered carbon atoms of the ring with striking regularity (v). This has raised the question of whether the ring is made not from acetate but from propionate [13]:



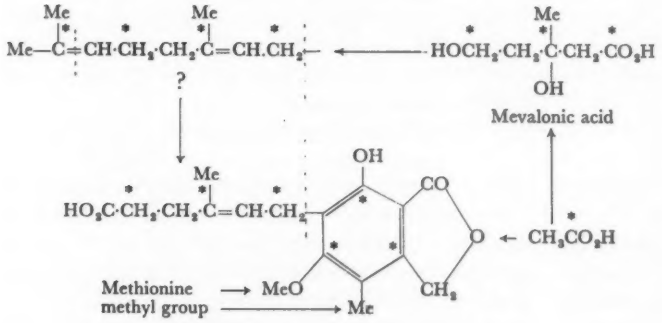
The question has not been finally answered, but it may be that here, too, acetate is the biogenetic unit and that methyl groups are added separately to the skeleton that it forms.

Work by Bu'Lock and Gregory suggests that modifications of β-keto acids formed from acetate may also lead to a family of antibiotics, produced by basidiomycetes, that contain acetylene links. It is evident, however, that the condensation of acetate in micro-organisms does not lead only to unbranched carbon chains. Three molecules of acetate can condense to yield mevalonic acid, a precursor of the isoprenoid units which make up the terpenoid groups of compounds:



Among the terpenoid hydrocarbons is squalene, which gives rise, after cyclization, to triterpenes and sterols. A few antibiotics from fungi, including helvolic acid and cephalosporin P, appear to be related to the sterol-triterpene family [14]; others contain terpenoid fragments. Thus mycophenolic acid (IX), which is produced by *Penicillium brevicompactum* and was first isolated in crystalline form in 1896, contains a terpenoid side-chain linked to an aromatic nucleus. Work with isotopically labelled compounds has shown that both the side-chain and the nucleus are formed from acetate, but that only the side-chain is formed from mevalonic acid [15]. The integration of two main series of reactions and the introduction of methyl groups from methionine are necessary, as illustrated in IX, for the synthesis of the complete skeleton.

The variety of the structures derived from acetate that have been found among the antibiotics is already remarkable and will doubtless be increased. However, other structural units appear to be employed in the synthesis of some of the new sugars or amino sugars that have been discovered in many of the antibiotics, including the macrolides, produced by species of *Streptomyces*. The skeletons of the streptidine, streptose, and *N*-methyl-L-glucosamine fragments of streptomycin (III) all contain six carbon atoms and can all be synthesized from D-glucose but not from acetate [16]. The guanidyl groups of the streptidine may be transferred to the molecule from arginine. The way in which these novel transformations of D-glucose are brought about has still to be determined, but it is likely that some of the reactions involved are used for the synthesis of molecules other than



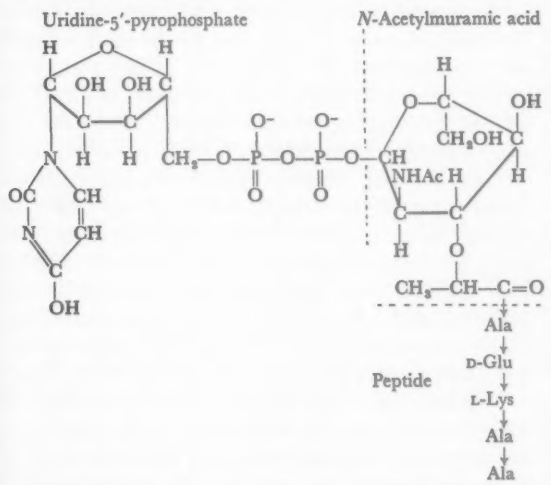
IX. Mycophenolic acid

streptomycin. Two other antibiotics, neomycin and kanamycin, have now been shown to contain a fragment that is structurally related to streptidine.

MECHANISMS OF ANTIBIOTIC ACTION

The antibiotics show a range of activity against living cells that is as varied as their chemical nature, but those that are used systemically in medicine are all of necessity much more toxic to certain micro-organisms than to animal tissues. The mechanisms by which these chemotherapeutic agents exert their selective toxicity differ, at least in detail, from one substance to another, and each presents its own specific problems. Minor changes in certain parts of the molecule, and particularly changes in configuration at asymmetric centres, often result in a virtual loss of activity. Nevertheless, the types of cellular processes with which the substances interfere seem to fall into a limited number of categories, and we are beginning to discern what some of these are.

The study of changes in bacteria that are brought about by certain antibiotics is now contributing to our knowledge of the nature of the outer layers of bacterial cells. This work began in 1949, when Park and Johnson found that three uridine nucleotides accumulated in staphylococci that had been treated for a short time with penicillin. More recently the main nucleotide has been shown to consist of uridine pyrophosphate linked, through the *N*-acetyl derivative of a new amino sugar, to a peptide containing D- and L-alanine, L-lysine, and D-glutamic acid. Its structure may be represented thus:



Meanwhile evidence had accumulated that the cytoplasm of Gram-positive bacteria was surrounded by a fragile membrane able to withstand a high internal osmotic pressure because it was supported by a rigid cell wall. When certain organisms are treated with the enzyme lysozyme in a hypertonic solution of sucrose, their walls dissolve and they are converted to spherical bodies known as protoplasts. The latter are bounded only by a cytoplasmic membrane, and they burst when the external osmotic pressure is reduced by dilution of the medium with water. The rigid walls can be separated from other cellular material, however, by mechanical disintegration of the organisms followed by centrifugation. When viewed with the electron microscope they appear as skins that have collapsed, but the outline of the cells from which they come is still discernible.

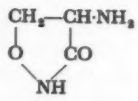
Analysis of the cell walls of Gram-positive bacteria has shown that they contain only a relatively small number of different amino acids. They also contain the new amino sugar, named muramic acid, that was first found in the uridine nucleotides that accumulate in staphylococci treated with penicillin. The proportions in which muramic acid, alanine, lysine, and glutamic acid occur in the cell walls of *Staphylococcus aureus* appear to be the same as those in which they occur in the nucleotide, and the only additional amino acid in the cell walls is glycine.

The discovery of this relationship has led to the hypothesis that the uridine nucleotide is an intermediate through which the muramic acid-peptide must pass in forming new cell wall material, and that the nucleotide accumulates in the presence of penicillin because its use is prevented by the action of the drug [17]. Two different considerations give plausibility to this suggestion. Firstly, uridine nucleotides are known in other cases to mediate the linking of sugars and of amino sugars to form more complex compounds. Secondly, there is independent and convincing evidence that penicillin can interfere with the synthesis of bacterial cell walls. When certain bacteria are allowed to grow in a hypertonic medium containing penicillin they are converted to protoplast-like bodies which lyse when the medium is diluted with water [18]. When penicillin is added to staphylococci in a medium containing glucose, glycine, alanine, glutamic acid, and lysine labelled with ^{14}C, the incorporation of the lysine into the cell wall is greatly inhibited [19].

The picture which thus emerges of penicillin disorganizing specifically the growth of bacterial

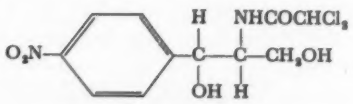
cell walls helps us to understand facts that were previously puzzling. The virtually complete lack of toxicity of penicillin to animal cells becomes understandable in principle, because these cells do not synthesize structures that are chemically similar to the walls of bacteria; both muramic acid and D-amino acids have so far been found only in micro-organisms. The fact that penicillin does not kill and lyse bacteria unless they are growing is explained if the formation of new cell wall is the only process affected, for it is not until the cell begins to grow that its existing wall becomes inadequate.

The synthesis of bacterial cell walls is evidently a complex process which could be inhibited selectively in more than one way. Several antibiotics have now been found to cause changes in staphylococci that resemble those produced by penicillin. Bacitracin brings about both a rapid accumulation of uridine nucleotides and the lysis of growing cells, and it inhibits the incorporation into the cell wall of amino acids labelled with ^{14}C [2, 19]. Similar, though less striking, changes are observed in the presence of D-cycloserine (x) [20]. Moreover, it is not only in bacteria that the cell walls may be a characteristic source of weakness. The chitinous walls of certain fungi appear to be damaged selectively by griseofulvin [21].



x. Cycloserine

Many antibiotics used in medicine exert no lytic action on sensitive bacteria, however, and the damage that they cause to the cells is quite different from that produced by penicillin or bacitracin. With a number of substances, including streptomycin, we are still uncertain about the type of cellular process that is primarily affected. But in the case of chloramphenicol and the tetracyclines much of interest has been learned [20]. These so-called 'broad spectrum' antibiotics all appear, despite the great difference in their chemical structures, to be inhibitors of the synthesis of bacterial protein. Chloramphenicol (the D-*threo* isomer of XI) is relatively specific in its action. In antibacterial concentrations it immediately inhibits the synthesis of protein in *Staphylococcus aureus* and *Escherichia coli*, but it does not inhibit the incorporation of amino acids labelled with ^{14}C into the cell wall, nor does it prevent the synthesis of nucleic acid. With the tetracyclines the picture is similar, but less clear-cut, for in high concentrations these substances interfere with several cellular processes.



XI. Chloramphenicol

It seems that amino acids are first activated, for biosynthesis of protein, by the formation of mixed anhydrides with adenylic acid, and are then in turn transferred to at least two types of ribonucleic acid before the specific pattern of peptide bonds is established. Chloramphenicol and the tetracyclines may interfere at different points in this sequence of events in bacterial cells. The stereochemical situation is interesting. An L-isomer of chloramphenicol has little effect on the synthesis of protein (from L-amino acids) but strongly inhibits the synthesis of a D-glutamyl polypeptide [20].

The ability of bacteria to acquire resistance to antibiotics is one of many examples of the adaptability of micro-organisms, and its study impinges on basic problems of protein and nucleic acid synthesis. Two related questions may be asked about resistant strains. By what mechanism are they formed, and how do they differ from their sensitive parents? An elegant procedure invented by Lederberg, whereby samples of a mass of bacteria are first transferred from the surface of a nutrient agar plate to a velvet pad and are then printed on to other plates to give replicas of the first, has left little doubt that bacteria highly resistant to streptomycin can be formed without any contact with the drug [22]. When successive prints are made from a normal plate of *E. coli* to plates containing streptomycin, most of the organisms fail to multiply. A small number, however, grow into colonies of resistant cells and the latter appear at congruent sites on different plates. This is readily intelligible only if resistant cells, formed by rare mutations, already exist on the normal plate and are later selected in the presence of the drug. But it is by no means certain that random mutation represents the only mechanism by which resistance to antibiotics develops.

Remarkably little is yet known about the specific biochemical differences that must exist between resistant bacteria and sensitive ones of the same species; but in a special case, of medical importance, the immediate cause of resistance is clearly established. A number of bacteria can

produce an enzyme, penicillinase, that brings about the rapid hydrolysis of penicillin. Among them are certain strains of staphylococci, and these strains may survive, while others are destroyed, during clinical use of the drug. The inherent ability to synthesize the enzyme appears to depend on a specific genetic character, but in some organisms the enzyme is normally present and in others its formation can be induced. In the latter, its rate of synthesis is greatly increased when penicillin is present in the medium. Penicillin is irreversibly bound by bacteria, probably near the cytoplasmic membrane, and the work of Pollock [23] has shown that with *B. cereus* only brief contact with the drug is required to establish a mechanism that will synthesize inducible penicillinase at a high and constant rate. The inducible enzyme appears to be identical with the constitutive enzyme formed by a mutant strain of the same organism. None of the available degradation products of penicillin show any power of induction. Nevertheless, the structural requirements of an inducer of the enzyme are not identical with those of a substrate of the enzyme. The penicillin-like antibiotic cephalosporin C is resistant to penicillinase, but induces its formation [24]. Although not a substrate, it combines with penicillinase and competitively inhibits the action of the enzyme on the true penicillins [25]. It remains to be seen whether a further member of the family can be found that has the specific action of a penicillin on cell-wall synthesis, but fails to activate the centre where penicillinase is formed, or that prevents the activation of this centre by penicillin while leaving cell-wall synthesis open to attack.

CONCLUSION

The main stream of biochemistry has been fed by studies on substances that are always found in living cells, such as carbohydrates, fats, proteins, and nucleic acids, and its progress has revealed that similar mechanisms are used in many different forms of life for the synthesis of essential structures and the provision of energy. The antibiotics appear at first sight to lie outside this widespread pattern. They are produced only by certain micro-organisms, and their antimicrobial activity is often highly selective. The biosynthesis and mode of action of these products might therefore be expected to illustrate the diversity rather than the unity of nature. In fact, this is only partly true.

What we now know, and can reasonably surmise, about the origin of the antibiotics points to the economy with which their novel and sometimes intricate structures are made. Amino acids and acetate, which feature as common structural units, have widespread functions in cellular metabolism. The condensation of acetate, as acetyl coenzyme A, to yield a β-keto acid, and of activated amino acids to form peptide chains, are familiar processes. So, too, is the transfer of a methyl group from *S*-adenosylmethionine to nitrogen, oxygen, or carbon in another molecule.

It may thus be only at certain stages in the synthesis of an antibiotic that enzymic mechanisms are involved that differ from the general patterns. Reactions in this category are responsible for the introduction of D-amino acids into peptide-like structures; for the intramolecular condensations that lead to penicillin, gliotoxin, and the tetracyclines; and for the building of conformations whose head-to-tail unions yield the large rings of the cyclopeptides and the macrolides. Some of them, however, can be regarded not as unique aberrations but as reflections of types of macromolecular organization that are shared by many species of micro-organism. D-Amino acids are commonly present in the cell walls of bacteria and actinomycetes, though they have not been detected in other forms of life. Large peptide and lactone rings, of a type previously unknown in natural products, have now been identified so often in the structures of antibiotics that their formation would seem to have a broad significance.

Our knowledge of the mode of action of the chemotherapeutic antibiotics parallels what we have learned about their biosynthesis. We can now define some of the main features of bacterial metabolism that are open to selective attack; one of these, the formation of cell walls, is a feature in which bacteria and actinomycetes differ from other kinds of cells. But the precise nature of the reactions that are inhibited has yet to be elucidated. So, too, have certain highly specific characteristics on which depend the degree of sensitivity to a given antibiotic. Why does the introduction of polar groups into the penicillin side-chain make the substance more active against some bacteria but much less active against others? Why does chloramphenicol inhibit protein synthesis more readily in bacteria than in animal cells? Factors, such as transport through cell membranes, which govern the accessibility of the drug to a sensitive structure may be concerned in some of these phenomena, but virtually nothing is yet known about them at the molecular level.

REFERENCES

[1] WOODRUFF, H. B. and MCDANIEL, L. E. in 'The Strategy of Chemotherapy'. Cambridge University Press, London. 1958.
[2] ABRAHAM, E. P. and NEWTON, G. G. F. in 'Amino Acids and Peptides with Antimetabolic Activity'. J. and A. Churchill Ltd, London. 1958.
[3] BROOKES, P., FULLER, A. T., and WALKER, J. *J. chem. Soc.*, 689, 1957.
[4] CRAIG, L. C., KONIGSBERG, W, and HILL, R. J. in 'Amino Acids and Peptides with Antimetabolic Activity'. J. and A. Churchill Ltd, London. 1958.
[5] ARNSTEIN, H. R. V. *Ann. Rep. chem. Soc.*, **54**, 339, 1957.
[6] BATCHELOR, F. R., DOYLE, F. P., NAYLOR, J. H. C., and ROLINSON, G. N. *Nature, Lond.*, **183**, 257, 1959.
[7] BELL, M. R., JOHNSON, J. R., WILDI, B. S., and WOODWARD, R. B. *J. Amer. chem. Soc.*, **80**, 1001, 1958.
[8] SUHADOLNIK, R. J. and CHENOWETH, R. G. *Ibid.*, **80**, 4391, 1958.
[9] JOHNSON, A. W. *Chem. Soc. Special Publ.* No. 5, 1956; BROCKMANN, H., BOHNSACK, G., FRANCK, B., GRÖNE, H., MUXFELDT, H., and SÜLING, C. H. *Angew. Chem.*, **68**, 70, 1956; KATZ, E. and GOSS, W. A. *Nature, Lond.*, **182**, 1668, 1958.
[10] BUTENANDT, A. in 'Perspectives in Organic Chemistry'. Interscience Publishers Inc., New York. 1956.
[11] ROBINSON, R. 'The Structural Relations of Natural Products'. Oxford University Press, London. 1955.
[12] BIRCH, A. J. *Progr. Chem. org. nat. Prod.*, **14**, 186, 1957.
[13] WOODWARD, R. B. *Angew. Chem.*, **68**, 13, 1956.
[14] ABRAHAM, E. P. 'Biochemistry of some Peptide and Steroid Antibiotics'. John Wiley & Sons Inc., New York. 1957.
[15] BIRCH, A. J., ENGLISH, R. J., MASSY-WESTROPP, R. A., and SMITH, H. *J. chem. Soc.*, 369, 1958.
[16] HUNTER, G. D. *J. Microbiol.*, **2**, 312, 1956.
[17] PARK, J. T. and STROMINGER, J. L. *Science*, **125**, 99, 1957.
[18] LEDERBERG, J. *Proc. nat. Acad. Sci. Wash.*, **42**, 574, 1956.
[19] PARK, J. T. *Biochem. J.*, **70**, 2P, 1958.
[20] HAHN, F. E. *Symp. No. 5, 4th Int. Congr. Biochem.*, 1958.
[21] BRIAN, P. W. *Ann. Bot. Lond.*, **13**, 59, 1949.
[22] LEDERBERG, J. and LEDERBERG, E. M. *J. Bact.*, **63**, 399, 1952.
[23] POLLOCK, M. R. *J. gen. Microbiol.*, **8**, 186, 1953.
[24] *Idem. Biochem. J.*, **66**, 419, 1957.
[25] ABRAHAM, E. P. and NEWTON, G. G. F. *Ibid.*, **63**, 629, 1956.

Book reviews

NUCLEAR SCIENCE

Annual Review of Nuclear Science, Vol. VIII, *edited by E. Segrè. Pp. vii+417. Annual Reviews Inc., Palo Alto, California.* 1958. $7.50.

This is in some way the mixture as before, but, even considering the high standard set by previous volumes, the ingredients this time are of quite unusual excellence. Those that were thrilled by Wick's recent review article on meson theory will not be disappointed with his 'Invariance Principles of Nuclear Physics'. It is a masterly account of what lies behind and beyond the great parity puzzle. Feshbach writes on the optical model and Segrè on antinucleons, each on the subject that is perhaps more than any other his own, and each does it very well. Of the other articles on physics, perhaps the most interesting is on hyperfragments, these strange nuclei in which one of the nucleons is replaced by a heavier baryon.

When the physicist turns to the other divisions he will find these of more interest to him than has been common in the past. The article on the control of radiation hazards in physics research is of great importance to him, and who could fail to find interest in 'Nuclear Astrophysics' or in 'Information Theory in Radiobiology', an attempt to account for the strange fact that radiation which does not affect more than one in 10^8 atoms in living tissue is enough to kill?

In this brief review it has been possible to refer to only seven of the thirteen articles that make up the volume, but the others are of equal variety and interest. L. R. B. ELTON

ORGANIC CHEMISTRY

A Text-book of Organic Chemistry, *by J. Read and F. D. Gunstone. Pp. xii+610. G. Bell & Sons Ltd, London.* 1958. 37*s.* 6*d. net.*

This work has been substantially rewritten, and, while the plan of the book remains essentially the same, the text has been thoroughly revised. The layout is now more attractive and readable.

The introductory historical survey tracing the rise of organic chemistry is brought up to date with a concise account of modern ideas of structure, including a short section on the orbital theory. The next section consists of an excellent account of methods of purification and analysis, and the use of micro-analysis is emphasized.

The major part of the work, dealing with the main classes of compound, is very well laid out; modern methods are given, and both formulae and reaction processes are attractively drawn. The authors have a happy knack of choosing good examples, and of giving interesting facts not usually available in a book of this size. The last few chapters deal neatly with more advanced topics, such as terpenes and alkaloids, in a manner which will encourage further reading. In addition there are sections on reaction mechanism and aromatic character which are brief, but excellent.

This book should be of particular value to senior boys in schools, as advanced topics are explained with a clarity and brevity suited to this level.

P. M. H. DAVIS

ORGANIC CHEMISTRY OF SULPHUR

Aspects of the Organic Chemistry of Sulphur, *by F. Challenger. Pp. vii+253. Academic Press Inc., New York; Butterworths Scientific Publications Ltd, London.* 1959. 40*s. net.*

Sulphur is the cinderella element of

organic chemistry: it is exploited, neglected, and rather despised by the many who pay homage to oxygen, nitrogen, or halogen, in a hydrocarbon frame. The redress which Professor Challenger brings is not the result of wand-waving but of a lifetime's devoted study. The aspects treated in his book are linked to the theme of the widespread and multiform occurrence of sulphur compounds in nature. The theme is not exhausted, such familiar topics as vitamin B_1, biotin, and penicillin being omitted, but excellent chapters are provided on natural sulphonium compounds (sulphides, sulphido-acids and sulphoxides are included), on mustard oils, on sulphur compounds from mineral sources, on biological methylation, and on the chemistry of co-enzyme A. Throughout the book emphasis is laid on the practical approach, and the first chapter offers the inexpert reader an introduction to the fundamental chemistry involved. Appropriately too, for this is a fascinating aspect, there is frequent discussion of the biochemical significance of some of the compounds. References appended to chapters and an adequate index complete the volume.

This book fills a need. It will be welcomed generally and can be consulted, read, or re-read with pleasure and with profit. J. D. LOUDON

CRYSTAL GROWTH

Growth and Perfection of Crystals, *edited by R. H. Doremus, B. W. Roberts, and D. Turnbull. Pp. xviii+609. John Wiley & Sons Inc., New York; Chapman and Hall Ltd, London.* 1958. £5 *net.*

This volume gives the proceedings of an international conference at Cooperstown, New York, on 27th–29th August 1958, and the editors must be most warmly congratulated on its prompt publication.

Many of the papers are directed to the problem of the origin and control of line imperfections in crystal growth; several of these papers deal with crystal whiskers, and discuss their dislocation content. Other papers describe mechanisms by which dislocations may be introduced into crystals, and two papers describe methods for growing large dislocation-free crystals of silicon and of silver bromide. The problem of growth from the melt may have been more closely defined as a result of the conference, which has emphasized the need for a better understanding of the nature of the liquid-solid interface. Dendrite formation, impurity effects, and polymer crystallization are also discussed. There is comparatively little about growth techniques.

The volume is expensive and bulky, but the bulkiness is the price that has to be paid for speedy publication. The contributed papers contain many striking photographs that show the beauty of recent techniques for the observation of dislocations, and the volume represents an authoritative conspectus of present knowledge. It is invaluable to any laboratory interested in crystal growth. R. V. JONES

BIOSYNTHESIS

A Ciba Foundation Symposium on Biosynthesis of Terpenes and Sterols, *edited by G. E. W. Wolstenholme and M. O'Connor. Pp. xii+311. J. & A. Churchill Ltd, London.* 1959. 45*s. net.*

The great virtue of these symposia and colloquia organized by the Ciba Foundation lies in bringing the leaders in a field together, and, this having been accomplished, the small and specialized audience brings out the best from the participants. Carefully prepared for publication, the results are unique summaries of current work and hypotheses. Without symposia such as these any attempt to get at grips with the subject would entail a well-nigh impossible task of reading original publications. This collection of seventeen papers deals with one of the 'growing points' of biochemistry and, as might be expected, the recurrent theme is that of mevalonic acid, the discovery of which some three years ago solved one of the problems in the biogenesis of steroids and, in doing so, stimulated many new lines of research.

It is perhaps a disadvantage inherent in the circumstances of presentation that a book such as this is not easy reading for those not themselves specialists. Possibly something might be done editorially to provide a remedy, even by commissioning an introductory article which would put the contributions in perspective and fit them into a scheme. In other ways something could certainly be done. For instance, in this volume surely the paper by Folkers and his associates on the discovery and elucidation of mevalonic acid should come first and the terse and allusive paper by Bloch, important as it is, should appear in a place where it would be less likely to deter the as yet uninitiated reader. As for the rest, the range is surprisingly wide: the application of some of the work to modern interest in hypercholesterolaemia will be clear: there is some complex terpene chemistry, the biogenesis of bile acids and carotenoids is dealt with, and enzyme problems are discussed at length. One might, in this last connection, quote one of the organic chemists: '. . . we have no right to assume that nature is using the methods of which we personally would approve.'

R. K. CALLOW

ZOOLOGY

Traité de zoologie: anatomie, systématique, biologie, *edited by P.-P. Grassé. Vol. XIII,* Agnathes et poissons —anatomie, éthologie, systématique. *Part I, pp. viii+924; part II, pp. viii+925–1812; part III, pp. vii+1813–2758. Masson et Cie, Paris.* 1958. *Each part, paper covers, fcs.* 12 000; *bound, fcs.* 13 000.

With the appearance, first of the ostracoderms and then of the jawed fishes, the possibility was opened for successful vertebrate exploitation of aquatic habitats and for the later evolution of terrestrial groups. Both possibilities were fully realized, giving these animals unique importance both in the range and success of modern fishes and in the indispensable evidence provided by fossil and archaic groups—recently dramatically reinforced by discovery of the coelacanth—on the course of vertebrate evolution. It is therefore reasonable that Volume XIII of this great treatise, undoubtedly the most important general account of zoology now being produced, should run to three parts containing a total of 2758 pages.

Twenty-two authors have contributed, some of them, notably the late Léon Bertin, being responsible for many sections. All are French with the exception of E. Stensio of Stockholm, to whom recourse has very rightly been made for the long and authoritative account of the ostracoderms, which follows a somewhat shorter description of modern cyclostomes by M. Fontaine. This account of the *Agnatha* occupies half of the first book.

The remaining contents of this part, and all of part II, consist of sections covering the various organ systems in all fishes. Descriptions of structure are admirably illustrated, the greatest care having clearly been taken to maintain a consistently high standard in line figures, half-tone reproductions (where alone some slight criticism might be

permitted), and occasional coloured plates. There is an accompanying account of function, either dealt with by the same author or separately. One is perhaps most impressed with the sections dealing with sense organs, electric organs (both structure and function), the endocrine system, and with reproduction in its many aspects. But throughout there is a wealth of well presented and illustrated information with only occasional gaps, such as lack of reference to the work of Gray and Harris on locomotion.

The last part deals initially with the living fish, first with growth, races, and migrations (these by L. Fage), and then with ecology and the broader aspects of geographical distribution. The long account of classification, dealing with both modern and fossil fishes, which occupies the greater part of this volume is preceded by an interesting account of the history of classification, illustrated with an X-ray photograph of mummified *Lates niloticus* and reproductions of the title pages of the Renaissance works of Belon and Rondelet. Finally comes a general description of the only living crossopterygian, *Latimeria*, by J. Millot and J. Anthony, illustrated with excellent photographs, including a coloured plate of one of the ten undamaged specimens obtained off Madagascar during three years of organized collecting. But despite its interest this is only one short section in a work of major importance unlikely to be approached in value, let alone superseded, for many years to come. C. M. YONGE

VERTEBRATE ANATOMY

Cours d'anatomie comparée des vertébrés, *by Jean G. Baer. Pp.* 206. *Masson et Cie., Paris*, 1958. *Fcs.* 5000.

As the preface of this book says, vertebrate comparative anatomy provides an author with a 'prodigious subject, too old ever to be out of date'. This, however, is no excuse for foisting upon readers a work consisting mostly of old knowledge, and that often capriciously selected and sometimes wrongly explained. There is a brief section on classification, followed by a concise treatment of each individual organ system as it appears in the different types of vertebrate. The treatment is, at best, sketchy, with many gaps. Colour change is stated to be only under hormonal control, with no mention of innervation of the chromatophores. More serious, there is almost no mention of muscles, though much about the skeleton. This is, of course, an old difficulty for vertebrate comparative anatomists, but something could have been said about recent developments in knowledge of mechanisms of movement, both in the water and by tetrapods. The entire central and peripheral nervous system is dealt with in nine pages, of which only three are devoted to the brain. On the other hand, there is an entire chapter of 19 pages for the sense organs, though in the course of this the author manages to describe the pineal organ of Agnatha as being 'without a structure comparable to a retina' and yet to give a picture so vague as to be compatible with this statement.

The figures are printed as a separate part of the book and are all based upon classical pictures from other books '*rehaussées par l'emploi de couleurs conventionelles, excitent le sens d'observation du lecteur*'. Unfortunately, in many of them there is little for him to observe, and labels have been mostly omitted so that he may concentrate on 'the idea or principle expressed and then turn for confirmation to the text'. But he must be cautious about what he finds there! J. Z. YOUNG

SPIDERS

The World of Spiders, *by W. S. Bristowe. Pp. xiii*+304. *Collins Publishers, London.* 1958. 30*s. net.*

Many excellent volumes have appeared in the New Naturalist series, and 'The World of Spiders' must surely rank among the best of these. Dr Bristowe has written the first full and authoritative general account of British spiders, based upon more than thirty-six years of observing and collecting in many parts of the world.

With fluent enthusiasm he recounts the role of spiders in folklore and literature, the history of British araneology, and the basic features of spider structure and behaviour, all interspersed with delightful anecdotes. The biology of each family is described in turn, and sufficient morphological detail is included to enable all the major genera to be recognized. Of particular interest is the chapter on 'The Origin of Spiders', in which a diphyletic origin of terrestrial Arachnida is postulated.

It is perhaps unfair to point out mistakes, especially when there are so few, but there are some minor misprints, and the dorsal spines of *Dysdera crocata* are on the fourth femur, not on the first.

No account of this book would be complete without mention of Arthur Smith's superb illustrations, whose accuracy reflects many hours of careful observation. This admirable book, which should be on every naturalist's shelf, will undoubtedly stimulate great interest in the study of spiders.

JOHN A. L. COOKE

ROCKET PROPELLENTS

Solid Propellent and Exothermic Compositions, *by James Taylor. Pp. vii*+153. *George Newnes Ltd, London.* 1959. 25*s. net.*

Dr Taylor has given a very readable account of the nature and uses of a wide variety of solid fuels: some liquid fuels are also discussed, although in less detail. A good deal of the work described was carried out by Dr Taylor and his colleagues in the Nobel Division laboratories of Imperial Chemical Industries. The titles of the chapters, which are reasonably self-contained and can be understood without detailed knowledge of the subjects considered, indicate the scope of the work. They are self-sustained exothermic chemical reactions; gunpowder and pyrotechnics; explosives; initiating explosives; propellents; low-temperature gas-producing reactions based on nitrites and the 'Hydrox' blasting device; ammonium nitrate, guanidine nitrate, and nitroguanidine compositions; rocket motors; solid charges for rockets and propulsion; power cartridges; and gasless reactions.

It is natural that a book of this kind should have a practical bias, but Dr Taylor has also stressed the need for fundamental work on solid state reactions. He has in fact indicated a number of problems which should be studied from this point of view.

This book contains information previously unpublished except as internal reports, and for this reason it will be of value to specialists in this field.

A. D. YOFFE

HISTORY OF CHEMISTRY

Robert Boyle and Seventeenth-century Chemistry, *by Marie Boas. Pp. viii*+240. *Cambridge University Press, London.* 1958. 30*s. net.*

By her study of the works of Boyle and a wide range of other chemical authors of or near his century, Miss Boas has rediscovered for herself, and here elaborates, the chief results of his labours in theoretical and experimental chemistry. She is, of course, by no means the first in modern times to do this with essentially the same

conclusions; but she insists sweepingly that all her precursors have seriously distorted the matter. Many of her *obiter dicta* outside chemistry itself are naive and superficial; and her cavalier dismissal of earlier authorities' assessments —for example, of 'The Sceptical Chymist'—are far too aggressively dogmatic. Such defects flaw the spirit in which any work of scholarship should be done. Boyle himself remarked: 'a man may be a champion for truth without being an enemy to civility.'

Her account of iatrochemistry is good; that of the doctrine of qualities less so; of Boyle's corpuscular ideas she rightly recognizes the deep importance. Her interpretation of his usages of the term 'element' differs from that of earlier exponents. Throughout the book the quotations from original sources are copious and aptly chosen. I. MASSON

HISTORY OF CYTOLOGY

A History of Cytology, *by Arthur Hughes. Pp. x+158. Abelard-Schuman Ltd, London.* 1959. 21*s. net.*

This useful book outlines the development of the major branches of cytology. The author necessarily relies heavily on the historical researches of others, especially in the large part of the work that is devoted to the cell theory, but he has made his own contribution as an historian, and he blends his findings with those of others in such a way that the story runs fluently. Interest is maintained throughout. The style is vigorous, clear, and free from pomposity. The book comes at an appropriate time, when many who know little of the background of the subject are turning to cytology as one invention after another provides new insight into the nature of the cell.

There are a few mistakes. The ten illustrations taken from Warneck show polar-body formation in *Limnaea* ('*Lymnaeus*'), not in *Limax*. Benda's iron-alizarin method does not involve the use of haematoxylin. Ergastoplasm was first named in a paper by M. and P. Bouin (not Garnier), and is now known not to be filamentous. The word chromatid is wrongly used. The distinguished zoologist G. C. Bourne is described as 'an Oxford botanist'. These, however, are minor blemishes in a book that deserves to be widely read by biologists. JOHN R. BAKER

HISTORY OF BOTANY

Species Plantarum, *by Carl Linnaeus.* A Facsimile. *Vol. I, pp. xiv+176+560. Vol. II, pp. xvi+640+148. The Ray Society, London. Vol. I,* 1957. 50*s. net. Vol. II,* 1959. 60*s. net.*

As a contribution to the quarter-millenary celebrations, in 1957, of Linnaeus's birth, the Ray Society undertook the publication of this facsimile of his most important work. A first volume which appeared in 1957 contained 560 of its 1200-odd pages, together with a 176-page introduction by W. T. Stearn. The introduction is designed to place the *Species Plantarum* in its full Linnean setting. This is well worth doing because the *Species Plantarum* is in fact closely integrated with Linnaeus's other work and is by no means the isolated opus it is sometimes taken to be. The project is now completed with a second volume containing the remainder of the facsimile itself, together with an appendix by W. T. Stearn and J. L. Heller clarifying Linnaeus's references to earlier authors.

The production of this facsimile is not merely an act of historical piety. The *Species Plantarum* is the internationally accepted starting point of modern botanical nomenclature, and as such is still a fundamental reference book. The original printing of 1753 was small, and surviving copies are collectors' pieces. Two previous facsimile printings in 1907 and 1934 have left the requirements of libraries and plant taxonomists still unsatisfied, and the present printing is designed first and foremost to fulfil a practical need. W. O. JAMES

HISTORY OF BRAIN STUDY

The History and Philosophy of Knowledge of the Brain and its Functions, *edited by F. N. L. Poynter. Pp. x+272. Blackwell Scientific Publications, Oxford.* 1958. 22*s.* 6*d. net.*

This volume includes almost all of the contributions to a three-day symposium held in London in the summer of 1957 under the auspices of the Wellcome Historical Medical Library. It could be maintained that the scope of the symposium was far too large, extending as it did from classical antiquity up to the present and ranging over a wide variety of cerebral and psychic functions. To the reviewer there is little of scientific interest in speculative attempts to describe the functioning of the brain when they are based upon the crude and often erroneous observations of past centuries. But, no doubt, the amazing compilation here presented should have a salutary influence in causing us to realize that even now our speculations on brain function are based upon very inadequate observations and may at some future time seem just as unwarranted as the speculations here assembled! Particularly valuable are the very erudite account of Descartes's ideas of brain function, and the several accounts by neurologists of ideas on brain function and consciousness with special reference to Hughlings Jackson. It is unfortunate that there is so little sign of integration, particularly as the subject is the brain, the integrator *par excellence*.

The book is very well produced, and there is a good index. J. C. ECCLES

HISTORY OF TECHNOLOGY

Man the Maker: A History of Technology and Engineering, *by R. J. Forbes. Pp. xv+365. Constable & Co. Ltd, London.* 1958. 30*s. net.*

The author, who is professor of the history of pure and applied sciences in antiquity at Amsterdam, first wrote this book for an American public in 1950. The new edition brings up to date the selective bibliography—which includes a handful of references to German and French sources—and has an extended epilogue on Technology and Progress. The development of 'man the maker' is traced from the time of the palaeolithic cave-dweller pondering the uses of fire to the United States citizen of our own generation, who supplements the physical strength of an easily wearied human frame from energy resources more than 2000 times as great *per caput* as were available in 1776.

It would be unreasonable to expect meticulous accuracy in a *tour de force* such as this not very long volume clearly represents. Instead, the reader will be grateful for an interesting and remarkably comprehensive narrative. The more discerning will also welcome the attempt, of which the technical expert commonly fights shy, to relate the record of invention and discovery which, since cities first rose on the banks of Euphrates and Nile, have so often changed tempo but never direction, to the far less certain chart of man's spiritual odyssey. Having shown how 'each new technique is born of the questions and needs of the times', Professor Forbes ends by asking: 'But were our questions, seen from the social angle, always the right ones?' The growth of technology has an often neglected bearing upon the problems of general history. T. K. DERRY

Notes on contributors

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Was born in 1924 and was educated at Dulwich College, London, and Queen's College, University of Oxford, where he read chemistry. After a year's research at Reading University he read biochemistry at the University of Cambridge, and then spent three years in the Biochemistry Department, University of Chicago. In 1951 he returned to Oxford as lecturer in biochemistry in the Department of Agriculture, where he has remained, with the exception of a year's sabbatical leave at Johns Hopkins University. His research has been mainly on the biochemistry of the mammary gland.

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Was born in London in 1927 and was educated at Marlborough College and King's College, Cambridge. After three years' research at the Department of Geodesy and Geophysics, Cambridge, he received the John Murray studentship for Oceanography and studied for a year at Columbia University, New York. In 1955 he was appointed a Senior Scientific Officer at the National Institute of Oceanography, where he is now working on the exploration of the floor of the deep oceans.

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Was born in Yorkshire in 1896 and was educated at Bradford Grammar School and Leeds University, where he graduated in civil engineering and gained a Ph.D. in geology. He was Lecturer and Reader in Applied Geology, Kings College, Durham University, until his retirement in 1956. His researches have been into the nature and constitution of coal and in mining and engineering technological history.

H. LUNDEGÅRDH,
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Was born in Stockholm in 1888. He studied at the University of Stockholm (Ph.D. 1912) and subsequently with Pfeffer in Leipzig and Klebs in Heidelberg. From 1916 to 1926 he was an assistant professor at the University of Lund. He was appointed professor of plant physiology and anatomy at the Agricultural Research Institute in *Experimentalfältet* (Stockholm) in 1926, and was head of the Institute of Plant Physiology at Ultuna-Upsala from 1937 to 1955. He established the Ecological Station at Hallands Väderö in 1917 and a laboratory for spectrophotometry at Penningby, Sweden, in 1947. His main research interest in the period 1928–40 was the development of spectrometric methods for studies in biochemistry and geochemistry.

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Was born in 1907 and educated at Charterhouse and at Caius College, Cambridge. He qualified in medicine at St Bartholomew's Hospital, London, and subsequently held a number of hospital posts. In 1949 he became a Nuffield Fellow in Genetical Research at Oxford University. He has conducted various expeditions in Africa and has also undertaken research in Brazil, Canada, and elsewhere. He is the author of numerous papers which deal with field ecology, in particular, with the rapid evolution of the black forms of certain species of moths, the phenomenon referred to as industrial melanism. He has also produced two scientific films demonstrating slow and rapid evolution in insects, his most recent one being 'Darwin and the Insect Adaptations of Brazil', which he made for the Darwin Centenary last year.

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